Steroids 77 (2012) 1177–1191

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Progesterone–adenine hybrids as bivalent inhibitors of P-glycoprotein-mediated multidrug efflux: Design, synthesis, characterization and biological evaluation

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ARTICLE INFO

Article history:
Received 23 March 2012
Received in revised form 5 July 2012
Accepted 15 July 2012
Available online 31 July 2012

Keywords:
Multidrug resistance
P-glycoprotein
Bivalent ligand
Adenine
Progesterone
PEG linker

ABSTRACT

Bivalent ligands were designed on the basis of the described close proximity of the ATP-site and the putative steroid-binding site of P-glycoprotein (ABCB1). The syntheses of 19 progesterone–adenine hybrids are described. Their abilities to inhibit P-glycoprotein-mediated daunorubicin efflux in K562/R7 human leukemic cells overexpressing P-glycoprotein were evaluated *versus* progesterone. The hybrid with a hexamethylene linker chain showed the best inhibitory potency. The efficiency of these progesterone-adenine hybrids depends on two main factors: (i) the nature of the linker and (ii) its attachment point on the steroid skeleton.

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1. Introduction

Chemotherapeutics are the most effective treatment for metastatic cancer, but their efficacy is limited by the multidrug resistance (MDR) phenotype that remains a significant impediment to successful cancer chemotherapy. MDR results from multifactor mechanisms [1]. A reduced intracellular drug accumulation is one of the prominent mechanisms of resistance in cancer cells, wherein drug-efflux pumps belonging to ABC superfamily of proteins are overexpressed [2]. One of these human ATP-dependant membrane transporters is P-glycoprotein (Pgp) which allows the efflux of a wide variety of structurally and functionally unrelated compounds [3]. Inhibition of Pgp may therefore improve chemotherapeutic treatments and numerous works [4] have focused on the development of Pgp modulators since the demonstration in the early 1980s of the reversal of resistance *in vitro* by Verapamil [5].

Pgp is also present in various normal cells [2a]. In particular, adrenal Pgp could play a role in secretion of steroid hormones [6]. Among these molecules, the more hydrophilic ones are transported by Pgp (e.g. cortisol) while the more hydrophobic ones function as inhibitors (e.g. progesterone) [7]. Moreover, progesterone and derivatives like progestagens (e.g. nomegestrone and megestrol) or antiprogestin RU 486 inhibit in some extend Pgp-mediated efflux of chemotherapeutic drugs such as vinblastine [7a,8], vincristine [6b], doxorubicin [9] or adriamycin [10]. Therefore, progesterone represents a lead compound for the design of Pgp inhibitors.

Pgp consists of two homologous halves, each containing six transmembrane domains (TMDs) and two nucleotide-binding domains (NBD1 and NBD2). NBD1 and NBD2 are separated by ~30 Å, as revealed by the X-ray crystallographic study of murine Pgp which has 87% sequence homology to human Pgp [11]. Pgp contains at least two transport-active binding sites within the TMDs. It remains unclear how steroidal compounds act at the molecular level. Shapiro et al. [12] have shown that progesterone could bind to a third allosteric binding site. Moreover, it has been postulated that a steroid-binding region would be adjacent to the ATP-binding site of Pgp [13]. Then, we decided to design and synthesize progesterone–adenine hybrids as potential bivalent ligands (Fig. 1) which may simultaneously bind to both sites of Pgp.

Bivalent ligands are known to often improve the binding affinities and selectivities compared to the monovalent counterparts

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http://dx.doi.org/10.1016/j.steroids.2012.07.010

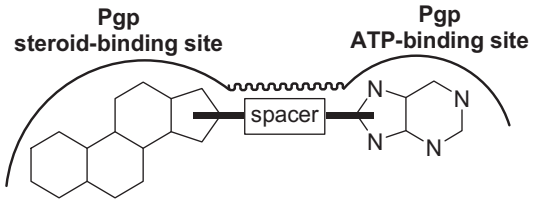


Fig. 1. Design of steroid-purine hybrids.

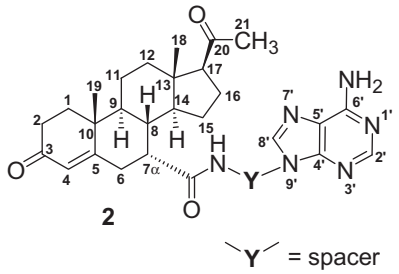


Fig. 3. Structures of C7-progesterone–adenine hybrids **2**. Numbering system for NMR data.

[14]. They can be used as tools to confirm the proximity of targeted binding sites [15]. This concept has been already applied to some homodimeric compounds targeting substrate binding sites of Pgp [16]. All these homodimers have been found to reverse the Pgp-mediated multiple drug resistance phenotype more efficiently than their respective monomers. For our part, we have recently designed new hetero-bivalent MDR modulators combining a steroid nucleus (progesterone) and a purine ring system (adenine) connected by a spacer. Progesterone was a known steroidal Pgp modulator [17] and adenine was first used as a mimic analogue of ATP because aromatic residues of the Pgp A-loop were known to interact with the adenine ring of ATP [18]. In order to evaluate our strategy, we have described the synthesis of C20-substituted progesterone derivatives **1** using an amide link as a connecting function and rather short-length spacers with different conformational flexibilities (Fig. 2) [19]. The Pgp-inhibition activity of these progesterone–adenine hybrids in leukemic MDR cells was similar to that of progesterone.

The structure of the linker and its attachment points on the two moieties (steroid and adenine) are important to consider when designing bivalent ligands. Therefore, we report here, on the one hand, the synthesis of two new C20-hybrids with flexible PEG linkers as Y and, on the other hand, the synthesis of ten C7-hybrids **2** (Fig. 3) with their respective abilities to inhibit Pgp-mediated daunorubicin efflux in K562/R7 human leukemic cells overexpressing P-glycoprotein. Our choice for the preparation of C7 derivatives was driven by literature precedents describing some C-7 substituted progesterone derivatives: they are known to possess an equivalent potency than cyclosporin A, a well-known Pgp modulator, while the most potent of them do not bind to progesterone receptors [9,20].

2. Experimental procedures

2.1. Material and methods

All commercially available chemicals and solvents were used as received. All air- and moisture-sensitive reactions were carried out under an argon atmosphere. All melting points were measured on a Barnstead Electrothermal 9200 apparatus and were uncorrected. ^{1}H spectra were recorded with Bruker ALS300 and Bruker DRX400 Fourier transform spectrometers, using an internal deuterium lock, operating at 300 MHz or 400 MHz, respectively. ^{13}C NMR spectra were recorded with a Bruker ALS300 and DRX400 Fourier transform spectrometers, using an internal deuterium lock, operating at 75 and 100 MHz, respectively. All spectra were recorded at 25 °C. Chemical shifts are reported in parts per million (ppm) relative to internal standard (tetramethylsilane, $\delta_H = 0.00$; $CDCl_3$, $\delta_H = 7.26$, $\delta_C = 77.36$; DMSO, $\delta_H = 2.54$, $\delta_C = 39.52$; CD_3OD, $\delta_H = 3.34$, $\delta_C = 49.86$). Carbon multiplicity was determined by DEPT experiments. Electron-Spray low-resolution mass spectra were recorded on a Thermo ALCQ Advantage spectrometer or Agilent 6120 spectrometer. High-resolution mass spectra were recorded on a Bruker MicroTOF Q or ThermoQuest FINNIGAN MAT 95 XL apparatus operating at 70 eV. UV spectra were recorded on a Shimadzu UV/Vis spectrometer UV-1700. Product purification was performed either by flash column chromatography using Merck Silicagel 60 Å (40–63 μm) or by preparative TLC carried out using Merck commercial glass plates (20 × 20 cm) coated (250 μm layer thickness) with Silicagel 60 F254 with visualization by ultraviolet. Analytical thin layer chromatography (TLC) was carried out using Merck commercial aluminum sheets coated (200 μm layer thickness) with Silicagel 60 F254, with visualization by ultraviolet or by spraying plates with diluted solution of H_2SO_4 or ninhydrine in ethanol followed by drying with heat gun. Flow cytometry analyses were carried out on a Beckton Dickinson FACS-Calibur.

2.2. Chemical syntheses

2.2.1. 6-(N-benzyloxycarbonylamino)hexan-1-ol (*4*)

To an ice-bath cooled solution of sodium carbonate (2.12 g, 20 mmol) and 6-aminohexan-1-ol **3** (1.17 g, 10 mmol) in water (30 mL) was added dropwise benzyl chloroformate (2.41 mL, 17 mmol). The reaction mixture was stirred overnight at room temperature then filtered. The precipitate was dissolved in dichloromethane and the organic layer was washed with water, dried over Na_2SO_4, filtered then concentrated *in vacuo* to give a solid

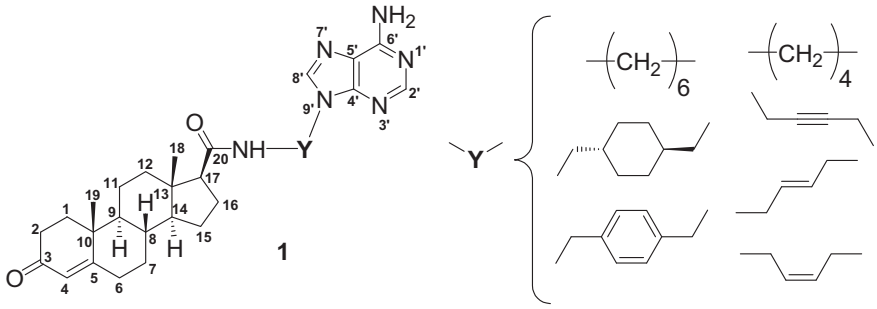


Fig. 2. Structure of progesterone–adenine hybrids **1** (C20-hybrids). Numbering system for NMR data.

which was recristallised from cyclohexane to give compound **4** as a white solid (1.98 g, 79% yield). Mp = 80–81 °C; ^{1}H NMR (DMSO-d_6) δ: 7.39–7.23 (m, 5H, 5 × H-phenyl), 7.21 (m, 1H, NH), 5.00 (s, 2H, C**H**$_2$Ph), 4.33 (t, 1H, J = 5.2 Hz, OH), 3.36 (q, 2H, J = 5.2 Hz, CH_2O), 2.97 (q, 2H, J = 6.6 Hz, CH_2N), 1.40–1.20 (m, 8H, 4 × CH_2) ppm; ^{13}C NMR (DMSO-d_6) δ: 156.9 (NHCOO), 138.18 (C-phenyl ipso), 129.19 (2 × C-phenyl meta, C-phenyl para), 128.56 (2 × C-phenyl ortho), 65.91 (O**C**H$_2$Ph), 61.50 (CH_2OH), 41.11 (CH_2), 33.34 (CH_2), 30.33 (CH_2), 27.02 (CH_2), 26.08 (CH_2) ppm; MS(ESI): m/z (%) 525 (70) $[2M+Na]^+$, 274 (83) $[M+Na]^+$, 252 (100) $[M+H]^+$; HRMS (ESI): m/z: calcd for $C_{14}H_{21}NO_3$ + Na: 274.1419; found: 274.1423.

2.2.2. [Trans-4-(N-benzyloxycarbonylaminomethyl) cyclohexyl] methanol (**7**)

To an ice-bath cooled suspension of lithium aluminum hydride (302 mg, 7.9 mmol) in anhydrous THF (20 mL) was added dropwise a suspension of *trans*-4-(aminomethyl)cyclohexylcarboxylic acid **5** (500 mg, 3.18 mmol) in anhydrous THF (20 mL). The reaction mixture was stirred at 0 °C for 1 h then it was heated under reflux for 3 h. The mixture was cooled to room temperature and quenched with water. The aqueous phase was extracted by dichloromethane. Addition of a few drops of aqueous solution of Rochelle salt avoids emulsion formation. Combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give [*trans*-4-(aminomethyl)cyclohexyl]methanol **6** as a yellow oil (320 mg, 70% yield) which should be used without delay.

To an ice-bath cooled suspension of compound **6** (320 mg, 2.23 mmol) and sodium carbonate (475 mg, 4.48 mmol) in water (20 mL) was added dropwise benzyl chloroformate (540 μL, 3.79 mmol). The reaction mixture was stirred at room temperature overnight then filtered. The precipitate was washed with water then recristallised from cyclohexane to give compound **7** as a white solid (432 mg, 70% yield). Mp = 95–96 °C; ^{1}H NMR ($CDCl_3$) δ: 7.40–7.30 (m, 5H, 5 × H-phenyl), 5.09 (s, 2H, C**H**$_2$Ph), 4.80 (bs, 1H, NH), 3.45 (d, 2H, J = 6.2 Hz, CH_2O), 3.05 (t, 2H, J = 6.3 Hz, CH_2N), 1.9–0.9 (m, 11H, 10 × H-cyclohexyl, OH) ppm; ^{13}C NMR ($CDCl_3$) δ: 156.7 (COO), 136.8 (C-phenyl ipso), 128.7 (2 × C-phenyl meta), 128.29 (2 × C-phenyl ortho), 128.26 (C-phenyl para), 68.6 (OCH_2), 66.8 (OCH_2), 47.4 (NCH_2), 40.6 (CH), 38.5 (CH), 30.1 (2 × CH_2 cyclohexyl), 29.0 (2 × CH_2 cyclohexyl) ppm; MS(ESI): m/z (%) 577 (100) $[2M+Na]^+$, 300 (65) $[M+Na]^+$; HRMS(ESI): m/z calcd for $C_{16}H_{23}NO_3$ + Na: 300.1570; found: 300.1577.

2.2.3. (4-Aminomethylphenyl)methanol (**9**) [21]

To a an ice-bath cooled suspension of lithium aluminum hydride (629 mg, 16.55 mmol) in anhydrous THF (40 mL), 4-(aminomethyl)benzoic acid **8** (1.0 g, 6.62 mmol) was added portion-wise. The reaction mixture was stirred at 0 °C for 1 h then it was heated under reflux for 3 h. The mixture was cooled to 0 °C and water was added. The aqueous phase was extracted with a mixture of dichloromethane/isopropanol (4:1). Addition of a few drops of aqueous solution of Rochelle salt avoids emulsion formation. Combined organic phases were dried over sodium sulfate, filtered and concentrated *in vacuo* to give compound **9** as a yellow solid (250 mg, 28%). ^{1}H NMR (CD_3OD) δ: 7.32 (m, 4H, H-phenyl), 4.58 (s, 2H, OCH_2), 3.79 (s, 2H, NCH_2) ppm.

2.2.4. 4-[(N-benzyloxycarbonylamino)methylphenyl]methanol (**10**)

To a suspension of compound **9** (240 mg, 1.75 mmol) and sodium carbonate (371 mg, 3.5 mmol) in water (20 mL) was added dropwise benzyl chloroformate (420 μL, 2.97 mmol). The reaction mixture was stirred at room temperature for 3 h then the aqueous phase was extracted with dichloromethane. The combined organic phases were washed with water, dried over Na_2SO_4, filtered, then concentrated *in vacuo* to give a solid, which was recristallised from ethyl acetate/cyclohexane to give compound **10** as a white solid (425 mg, 90% yield). Mp = 112–113 °C; ^{1}H NMR ($CDCl_3$) δ: 7.40–7.20 (m, 9H, 9 × H-phenyl), 5.14 (s, 2H, C**H**$_2$OCONH), 5.04 (m, 1H, NH), 4.68 (d, 2H, J = 4.4 Hz, C**H**$_2$OH), 4.38 (d, 2H, J = 5.8 Hz, C**H**$_2$NH), 1.61 (m, 1H, OH) ppm; ^{13}C NMR ($CDCl_3$) δ: 156.54 (COO), 140.36 (C-phenyl ipso), 137.99 (C-phenyl ipso), 136.58 (C-phenyl ipso), 128.69 (CH-phenyl), 128.32 (CH-phenyl), 128.30 (CH-phenyl), 127.89 (CH-phenyl), 127.47 (CH-phenyl), 67.05 (CH_2), 65.17 (CH_2), 45.02 (CH_2) ppm; MS(ESI): m/z (%) 564 (18) $[2M+Na]^+$, 294 (100) $[M+Na]^+$; HRMS(ESI): m/z calcd for $C_{16}H_{17}NO_3$ + Na: 294.1101; found: 294.1092.

2.2.5. 4-(Mesyloxy)but-2-yn-1-ol (**12**)

To an ice-bath cooled solution of but-2-yne-1,4-diol **11** (2.58 g, 30 mmol) in anhydrous THF (35 mL) were added dropwise methanesulfonyl chloride (2.30 mL, 30 mmol) and triethylamine (4.20 mL, 30 mmol). After the addition, the reaction mixture was allowed to warm up to room temperature. After 17 h stirring, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/methanol, 97:3) to give compound **12** as a colorless oil (2.23 g, 45% yield). ^{1}H NMR ($CDCl_3$) δ: 4.89 (t, 2H, J = 1.8 Hz, C**H**$_2$OMs), 4.34 (m, 2H, C**H**$_2$OH), 3.12 (s, 3H, CH_3), 1.92 (m, 1H, OH) ppm.

2.2.6. 4-(N-tert-butyloxycarbonylamino)but-2-yn-1-ol (**14**) [22]

Compound **12** (1.02 g, 6.02 mmol) was stirred in ammonium hydroxide (15 mL) for 1 h then the solvent was evaporated. The residue was treated with Dowex 1X8 $R_3N^+Cl^-$ prewashed with 4% NaOH aq. solution. The filtrate was concentrated and dried under vacuum to give 4-aminobut-2-yn-1-ol **13** as a yellow solid (880 mg, 76% yield) which should be used without delay. Mp = 58 °C (lit. 59–60 °C) [23]; ^{1}H NMR ($CDCl_3$) δ: 4.22 (t, 2H, J = 1.9 Hz, CH_2O), 3.45 (t, 2H, J = 1.9 Hz, CH_2N), 3.42 (s, 1H, OH), 2.50 (s, 2H, NH_2) ppm.

To a solution of compound **13** (65 mg, 7.64 mmol) and di-*tert*-butyl dicarbonate (1.67 g, 7.66 mmol) in anhydrous THF (30 mL), was added dropwise triethylamine (1.08 mL, 7.69 mmol) at 0 °C. The mixture was stirred overnight at room temperature then, the solvent was evaporated and the residue was dissolved in dichloromethane. The organic phase was washed twice with water and the aqueous phase was extracted by dichloromethane. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give compound **14** as a colorless oil (1.03 g, 73% yield). ^{1}H NMR ($CDCl_3$) δ: 5.18 (bs, 1H, NH), 4.18 (d, 2H, J = 5.2 Hz, CH_2O), 3.88 (d, 2H, J = 5.2 Hz, CH_2N), 3.71 (m, 1H, OH), 1.38 (s, 9H, $(CH_3)_3C$) ppm.

2.2.7. 9-[(6-N-benzyloxycarbonylamino)hexyl]adenine (**16**)

To an ice-bath cooled solution of compound **14** (260 mg, 1.40 mmol) in anhydrous THF (5 mL), were added dropwise methanesulfonyl chloride (130 μL, 1.68 mmol) and triethylamine (240 μL, 1.71 mmol). After the addition, the reaction mixture was allowed to warm up to room temperature. After 17 h stirring, the reaction mixture was filtered then concentrated *in vacuo* and the residue was dissolved in dichloromethane. The organic layer was washed with water, dried over Na_2SO_4, filtered then concentrated *in vacuo* to give compound **15** as a yellow oil (380 mg, 76% yield) which should be used without delay.

To a suspension of adenine (1.61 g, 11.9 mmol) and triphenylphosphine (2.34 g, 8.9 mmol) in dry THF (10 mL) under argon atmosphere, was added a solution of compound **4** (1.74 g, 6.9 mmol) and diisopropylazadicarboxylate (1.77 mL, 8.9 mmol) in dry THF (10 mL). The reaction mixture was stirred at room temperature overnight then the solvent was evaporated. The precipitate was washed with a mixture dichloromethane/methanol (90:10) then combined filtrates were concentrated *in vacuo*. The

residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give a crude material which was recristallised from methanol to give compound **16** as a white solid (1.05 g, 41% yield). Mp = 132–133 °C; UV ($CHCl_3$) λ_{max} 261 nm; ^{1}H NMR (DMSO-d_6) δ: 8.15 (s, 1H, H-2), 8.13 (s, 1H, H-8), 7.40–7.10 (m, 8H, 5 × H-phenyl, NH_2, NHCOO), 4.99 (s, 2H, C**H**$_2$Ph), 4.11 (t, 2H, J = 7 Hz, CH_2N), 2.96 (q, 2H, J = 6.3 Hz, C**H**$_2$NH), 1.77 (m, 2H, CH_2), 1.45–1.15 (m, 6H, 3 × CH_2) ppm; ^{13}C NMR (DMSO-d_6) δ: 156.93 (NHCOO), 156.82 (C-6), 153.21 (C-2), 150.39 (C-4), 141.67 (C-8), 138.16 (C-phenyl ipso), 129.17 (2 × C-phenyl meta, C-phenyl para), 128.56 (2 × C-phenyl ortho), 119.61 (C-5), 65.93 (**C**H$_2$Ph), 43.67 (CH_2), 40.98 (CH_2), 30.21 (CH_2), 30.07 (CH_2), 26.56 (CH_2), 26.51 (CH_2) ppm; MS(ESI): *m/z* (%) 758 (13) $[2M+Na]^+$, 369 (100) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{19}H_{24}N_6O_2$ + H: 369.2039; found 369.2035.

2.2.8. 9-(6-Aminohexyl)adenine (**17**)

To a solution of compound **16** (950 mg, 2.58 mmol) in methanol (50 mL) was added palladium on carbon (400 mg, 10 wt.%). The reaction mixture was maintained under H_2 atmosphere (1 atm) for 1 h. The resulting mixture was filtered over Celite and the filtrate was concentrated *in vacuo* to give compound **17** as a white solid (600 mg, 100% yield). Mp = 160–161 °C; UV ($CHCl_3$) λ_{max} 261 nm; ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 1H, H-2), 8.12 (s, 1H, H-8), 7.17 (s, 2H, NH_2), 4.11 (t, 2H, J = 7.1 Hz, CH_2N), 2.93 (bs, 2H, NH_2), 2.47 (m, 2H, C**H**$_2$NH$_2$), 1.78 (qn, 2H, J = 7.2 Hz, CH_2), 1.35–1.15 (m, 6H, 3 × CH_2) ppm; ^{13}C NMR (DMSO-d_6) δ: 155.94 (C-6), 152.33 (C-2), 149.54 (C-4), 140.83 (C-8), 118.73 (C-5), 42.83 (CH_2), 41.37 (CH_2), 32.91 (CH_2), 29.39 (CH_2), 25.92 (CH_2), 25.79 (CH_2) ppm; MS(CI): *m/z* (%) 235 (100) $[M+H]^+$; HRMS (CI): *m/z*: calcd for $C_{11}H_{18}N_6$ + H: 235.1671; found: 235.1671.

2.2.9. 9-[Trans-4-(N-benzyloxycarbonylaminomethyl)cyclohexylmethyl]adenine (**18**)

To an ice-cooled solution of triphenylphosphine (993 mg, 3.79 mmol) in anhydrous THF (15 mL), was added dropwise diisopropylazadicarboxylate (750 μL, 3.79 mmol). The resulting mixture was stirred for 30 min at room temperature then it was added dropwise to a suspension in anhydrous THF (3 mL) of adenine (511 mg, 3.79 mmol) and compound **7** (350 mg, 1.26 mmol). The reaction mixture was stirred at room temperature overnight then filtered. The precipitate was washed with a mixture dichloromethane/methanol (90:10) then combined filtrates were concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give compound **18** as a white solid (287 mg, 49 % yield). Mp = 229 °C; ^{1}H NMR (DMSO-d_6) δ: 8.12 (s, 1H, H-2), 8.08 (s, 1H, H-8), 7.37–7.21 (m, 5H, 5 × H-phenyl), 7.16 (s, 2H, NH_2), 4.98 (s, 2H, C**H**$_2$-Ph), 3.97 (d, 2H, J = 7.0 Hz, CH_2N), 2.82 (t, 2H, J = 6.1 Hz, C**H**$_2$NHCbz), 1.80–0.70 (m, 10H, 10 × H-cyclohexyl) ppm; ^{13}C NMR (DMSO-d_6) δ: 156.23 (NHCOO), 155.94 (C-6), 152.36 (C-2), 149.72 (C-4), 141.20 (C-8), 137.30 (C-phenyl ipso), 128.32 (2 × C-phenyl meta, C-phenyl para), 127.70 (2 × C-phenyl ortho), 118.64 (C-5), 65.09 (**C**H$_2$Ph), 48.72 (CH_2), 46.46 (CH_2), 29.50 (2 × CH_2), 29.40 (2 × CH_2), 37.67 (2 × CH) ppm; MS(ESI): *m/z* (%) 417 (36) $[M+Na]^+$, 395 (6) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{21}H_{26}N_6O_2$ + Na: 417.2015; found: 417.2012.

2.2.10. 9-(Trans-4-aminomethylcyclohexylmethyl)adenine (**19**)

To a solution of compound **18** (255 mg, 0.65 mmol) in methanol (20 mL) was added palladium on carbon (100 mg, 10 wt.%). The reaction mixture was maintained under pressure of H_2 (5 atm) for 15 min. The resulting mixture was filtered over Celite and the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC (CH_2Cl_2/MeOH/$NH_4OH_{aq.}$, 80:20:1) to give compound **19** as a white solid (120 mg, 71% yield). Mp (dec.); UV ($CHCl_3$) λ_{max} 261 nm; ^{1}H NMR (DMSO-d_6) δ: 8.12 (s, 1H, H-2), 8.10 (s, 1H, H-8), 7.61(bs, 2H, NH_2), 7.18 (s, 2H, NH_2), 3.98 (d, 2H, J = 7.1 Hz, CH_2N), 2.59 (d, 2H, J = 7.1 Hz, C**H**$_2$NH$_2$), 1.89–1.68 (m, 3H, 3 × H-cyclohexyl), 1.60–1.44 (m, 3H, 3 × H-cyclohexyl), 1.05–0.78 (m, 4H, 4 × H-cyclohexyl) ppm; ^{13}C NMR (DMSO-d_6) δ: 155.93 (C-6), 152.36 (C-2), 149.70 (C-4), 141.24 (C-8), 118.64 (C-5), 48.61 (CH_2), 44.17 (CH_2), 29.14 (2 × CH_2), 28.92 (2 × CH_2), 37.29 (CH), 35.37 (CH) ppm; MS(CI): *m/z* (%) 261 (100) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{13}H_{20}N_6$ + H: 261.1828; found: 261.1826.

2.2.11. 9-[4-(N-benzyloxycarbonylaminomethyl)phenylmethyl] adenine (**20**)

To an ice-bath cooled solution of triphenylphosphine (1.16 g, 4.42 mmol) in anhydrous THF (18 mL), was added dropwise diisopropylazadicarboxylate (880 μL, 4.44 mmol). The resulting mixture was stirred for 30 min at room temperature. Adenine (598 mg, 4.43 mmol) and a solution of compound **10** (400 mg, 1.47 mmol) in anhydrous THF (10 mL) were then added. The reaction mixture was stirred at room temperature overnight then filtered. The precipitate was washed with a mixture dichloromethane/methanol (90:10) then combined filtrates were concentrated *in vacuo*. The residue was purified by preparative TLC (CH_2Cl_2/MeOH, 95:5) to give compound **20** as a white solid (100 mg, 18% yield). Mp = 227–228 °C; ^{1}H NMR (DMSO-d_6) δ: 8.26 (s, 1H, H-2), 8.16 (s, 1H, H-8), 7.79 (t, 1H, J = 6.1 Hz, NH), 7.48–7.18 (m, 11H, 9 × H-phenyl + NH_2), 5.33 (s, 2H, CH_2N), 5.01 (s, 2H, C**H**$_2$OCONH), 4.15 (d, 2H, J = 6.1 Hz, C**H**$_2$NH) ppm; ^{13}C NMR (DMSO-d_6) δ: 156.31(NHCOO), 155.58 (C-6), 152.09 (C-2), 149.36 (C-4), 140.95 (C-8), 139.32 (C-phenyl ipso), 137.12 (C-phenyl ipso), 135.58 (C-phenyl ipso), 128.33 (CH-phenyl), 127.78 (CH-phenyl), 127.74 (CH-phenyl), 127.56 (CH-phenyl), 127.34 (CH-phenyl), 118.64 (C-5), 65.37 (**C**H$_2$OCO), 45.96 (CH_2), 43.50 (CH_2) ppm; MS(ESI): *m/z* (%) 798 (72) $[2M+Na]^+$, 776 (17) $[2M+H]^+$, 411 (29) $[M+Na]^+$, 389 (100) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{21}H_{20}N_6O_2$ + H: 389.1721; found: 389.1705.

2.2.12. 9-(4-aminomethylphenylmethyl)adenine (**21**)

To a solution of compound **20** (80 mg, 0.20 mmol) in methanol (30 mL) and ethylacetate (30 mL) was added palladium on carbon (80 mg, 10 wt.%). The reaction mixture was maintained under pressure of H_2 (13 atm) overnight. The resulting mixture was filtered over Celite and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2/MeOH 90/10 then CH_2Cl_2/MeOH/$NH_4OH_{aq.}$, 90:10:2) to give compound **21** as a white solid (40 mg, 77% yield). Mp (dec.); ^{1}H NMR (CD_3OD) δ: 8.20 (s, 1H, H-2), 8.18 (s, 1H, H-8), 7.41–7.18 (m, 6H, H-phenyl + NH_2), 5.45 (s, 2H, CH_2N), 3.98 (s, 2H, C**H**$_2$NH$_2$) ppm; ^{13}C NMR (CD_3OD) δ: 157.38 (C-6), 153.91 (C-2), 150.68 (C-4), 142.59 (C-8), 138.04 (C-phenyl ipso), 137.44 (C-phenyl ispo), 130.07 (CH-phenyl), 129.35 (CH-phenyl), 120.00 (C-5), 47.72 (CH_2), 44.66 (CH_2) ppm; MS(ESI): *m/z* (%) 255 (100) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{13}H_{14}N_6$ + H: 255.1353; found: 255.1342.

2.2.13. 9-[(4-N-tert-butyloxycarbonylamino)but-2-ynyl]adenine (**22**)

A suspension of adenine (380 mg, 2.8 mmol) and potassium carbonate (233 mg, 1.68 mmol) in DMF (40 mL) was heated to 75 °C for 1 h. To the resulting mixture was added a solution of compound **15** (370 mg, 1.4 mmol) in DMF (10 mL) and dichloromethane (1 mL). Stirring was carried on at 75 °C for 1h30 then solvents were removed under vacuum. The residue was partitioned between water and dichloromethane. Aqueous phase was extracted by CH_2Cl_2 and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 95:5) to give compound **22** as a white solid (320 mg, 75% yield). Mp = 200 °C (dec.); ^{1}H NMR (DMSO-d_6) δ: 8.18 (s, 1H, H-2), 8.15 (s, 1H, H-8), 7.26 (s, 2H, NH_2), 5.00 (s, 2H, CH_2N), 3.75 (d, 2H, J = 5.3 Hz, C**H**$_2$NHCOO), 1.36 (m, 10H, NH, $(CH_3)_3$C) ppm; ^{13}C NMR (DMSO-d_6) δ: 155.99 (C-6),

155.23 (NHCOO), 152.64 (C-2), 149.06 (C-4), 140.03 (C-8), 118.54 (C-5), 82.61 (**C**≡C), 78.27 (C≡**C**), 75.79 (**C**$(CH_3)_3$), 34.47 (CH_2), 29.50 (CH_2), 28.15 ($3 \times CH_3$) ppm; MS(CI): *m/z* (%) 303 (100) $[M+H]^+$; HRMS (CI): *m/z*: calcd for $C_{14}H_{18}N_6O_2$ + H: 303.1569; found: 303.1568.

*2.2.14. 9-(4-Aminobut-2-ynyl)adenine (**23**)*

To a suspension of compound **22** (280 mg, 0.93 mmol) in water (15 mL) was added dropwise concentrated HCl (3 mL) under vigorous stirring. The reaction mixture was stirred for few minutes then solvent was removed. The residue was dissolved in water and treated with Dowex 1X8 $R_3N^+Cl^-$ prewashed with 4% NaOH aq. Solution. The filtrate was concentrated and dried *in vacuo* to give compound **23** as a white solid (150 mg, 80% yield) which was recristallised from methanol. Mp = 135 °C (dec.); ^{1}H NMR (DMSO-d_6) δ: 8.24 (s, 1H, H-2), 8.16 (s, 1H, H-8), 7.76 (bs, 2H, NH_2), 7.29 (s, 2H, NH_2), 5.10 (m, 2H, CH_2N), 3.71 (m, 2H, C**H**$_2NH_2$) ppm; ^{13}C NMR (CD_3OD) δ: 157.34 (C-6), 153.88 (C-2), 150.20 (C-4), 142.04 (C-8), 119.93 (C-5), 86.94 (**C**≡C), 76.26 (C≡**C**), 34.22 (CH_2), 31.41 (CH_2) ppm; MS(CI): *m/z* (%) 203 (100) $[M+H]^+$; HRMS (CI): *m/z*: calcd for $C_9H_{10}N_{6+H}$: 203.1045; found: 203.1043.

*2.2.15. (E)-9-(4-aminobut-2-enyl)adenine (**24**) [24]*

To a suspension of compound **23** (200 mg, 1 mmol) in anhydrous THF (10 mL) was added lithium aluminum hydride (400 mg, 20 mmol). The mixture was stirred for two days at room temperature then the reaction was quenched by adding an aqueous solution of sodium hydroxide (1N, 5 mL). The precipitate was filtered, and washed with ethyl acetate. Combined filtrates were concentrated *in vacuo* then purified by flash chromatography on silica gel (CH_2Cl_2/MeOH/$NH_4OH_{aq.}$, 20:20:1) to give compound **24** as a white solid (57 mg, 29% yield). UV ($CHCl_3$) λ_{max} 260 nm; ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 1H, H-2), 8.10 (s, 1H, H-8), 7.21 (s, 2H, NH_2), 5.84–5.64 (m, 2H, HC = CH), 4.73 (d, 2H, J = 5.4 Hz, CH_2N), 3.40–3.10 (m, 4H, C**H**$_2NH_2$, NH_2) ppm; ^{13}C NMR (DMSO-d_6) δ: 155.94 (C-6), 152.46 (C-2), 149.32 (C-4), 140.53 (C-8), 135.54 (**CH** = CH), 123.61 (CH = **CH**), 118.65 (C-5), 44.11 (CH_2), 42.52 (CH_2) ppm.

*2.2.16. (Z)-9-(4-aminobut-2-enyl)adenine (**25**) [24]*

To a solution of compound **23** (70 mg, 0.35 mmol) in methanol (70 mL) was added Lindlar catalyst (7 mg, 5 wt.%) and two drops of quinoline. The reaction mixture was maintained under H_2 atmosphere (1 atm) until the reaction was complete (TLC control). Then the reaction mixture was filtered over Celite and the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC (CH_2Cl_2/MeOH/$NH_4OH_{aq.}$, 30:20:1) to give compound **25** as a white solid (47 mg, 67% yield). ^{1}H NMR (CD_3OD) δ: 8.17 (s, 1H, H-2), 8.09 (s, 1H, H-8), 5.85–5.60 (m, 2H, HC = CH), 5.00–4.70 (m, 6H, CH_2N, $2 \times NH2$), 3.53 (d, 2H, J = 6.8 Hz, C**H**$_2NH_2$) ppm; ^{13}C NMR (CD_3OD) δ: 157.28 (C-6), 153.70 (C-2), 150.46 (C-4), 142.34 (C-8), 134.46 (**CH** = CH), 126.19 (CH = **CH**), 120.01 (C-5), 41.45 (CH_2), 38.58 (CH_2) ppm.

*2.2.17. 9-(4-aminobutyl)adenine (**26**)*

To a solution of compound **23** (250 mg, 1.24 mmol) in methanol (70 mL) was added palladium on carbon (200 mg, 10 wt.%). The reaction mixture was maintained under H_2 atmosphere (1 atm) until the reaction was complete (TLC control). Then the reaction mixture was filtered over Celite and the filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH/$NH_4OH_{aq.}$, 20:20:1) to give compound **26** as a white solid (227 mg, 90% yield). Mp = 164–166 °C; ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, H-2), 8.12 (s, 1H, H-8), 7.17 (s, 2H, NH_2), 4.12 (t, 2H, J = 7.3 Hz, CH_2N), 1.81 (qn, J = 7.5 Hz, 2H, C**H**$_2NH_2$), 1.28 (qn, J = 6.9 Hz, 4H, $2 \times CH_2$) ppm; ^{13}C NMR (DMSO-d_6) δ: 155.93 (C-6), 152.33 (C-2), 149.54 (C-4), 140.83 (C-8), 118.73 (C-5), 42.85 (CH_2), 41.08 (CH_2), 30.26 (CH_2), 26.99 (CH_2) ppm; MS(CI): *m/z* (%) 207 (100) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_9H_{14}N_6$ + H: 207.1358; found: 207.1361.

*2.2.18. N-[2-(2-hydroxyethoxy)ethyl]phtalimide (**28a**) [25]*

To a solution of chloroethoxyethanol **27a** (623 mg, 5 mmol) in DMF (5 mL) was added potassium phtalimide (770 mg, 4.16 mmol). The reaction mixture was then stirred for 2 h at 130 °C. After addition of water (10 mL) and EtOAc (20 mL) and decantation, the aqueous phase was extracted with EtOAc (2×20 mL) and the organic phase was dried over Na_2SO_4. After filtration and removal of the solvents under reduced pressure, the residue was purified by flash chromatography (Cyclohexane/Ethyl acetate, 30:70) to afford compound **28a** (836 mg, 85%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.84 (m, 2H, $2 \times$ H-phenyl), 7.71 (m, 2H, $2 \times$ H-phenyl), 3.90 (t, 2H, J = 5.4 Hz, CH_2), 3.73 (m, 2H, CH_2), 3.67 (m, 2H, CH_2), 3.60 (m, 2H, CH_2), 2.32 (broad s, 1H, OH) ppm.

*2.2.19. N-{2-[2-(2-Hydroxyethoxy)ethoxy]ethyl}phtalimide (**28b**) [26]*

To a solution of chloroethoxy(ethoxy)ethanol **27b** (1.09 g, 6.47 mmol) in DMF (7 mL) was added potassium phtalimide (1 g, 5.39 mmol). The reaction mixture was then stirred for 2 h at 130 °C. After addition of water (10 mL) and EtOAc (20 mL) and decantation, the aqueous phase was extracted with EtOAc (3×20 mL) and the organic phase was dried over Na_2SO_4. After filtration and removal of the solvents under reduced pressure, the residue was purified by flash chromatography (Cyclohexane/Ethyl acetate, 30:70) to afford compound **28b** (1.46 g, 97%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.85 (m, 2H, $2 \times$ H-phenyl), 7.71 (m, 2H, $2 \times$ H-phenyl), 3.91 (t, 2H, J = 5.7 Hz, CH_2), 3.75 (t, 2H, J = 5.6 Hz, CH_2), 3.58–3.67 (m, 6H, $3 \times CH_2$), 3.53 (m, 2H, CH_2), 2.53 (broad s, 1H, OH) ppm.

*2.2.20. N-[2-(2-iodoethoxy)ethyl]phtalimide (**29a**) [25]*

To a solution of compound **28a** (1 g, 4.25 mmol) in Et_2O/MeCN (13.5 mL/4.5 mL) at 0 °C was added imidazole (868 mg, 12.7 mmol), PPh_3 (1.67 g, 6.37 mmol) and I_2 (1.62 g, 6.37 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 1 h. After addition of pentane, the resulting mixture was filtered off on a sintered glass funnel filled with a pad of silica gel 1 cm thick, and washed with EtOAc. After evaporation of the filtrate under reduced pressure, the residue was purified by flash chromatography (Cyclohexane/Ethyl acetate, 70:30) to afford compound **29a** (1.06 g, 72%) as a yellow solid. Mp = 84–85 °C (lit. 79–81 °C) [24]; ^{1}H NMR ($CDCl_3$) δ: 7.85 (m, 2H, $2 \times$ H-phenyl), 7.71 (m, 2H, $2 \times$ H-phenyl), 3.90 (t, 2H, J = 5.1 Hz, CH_2), 3.73 (m, 4H, CH_2), 3.18 (t, 2H, J = 6.6 Hz, CH_2) ppm.

*2.2.21. N-{2-[2-(2-iodoethoxy)ethoxy]ethyl}phtalimide (**29b**) [27]*

To a solution of compound **28b** (1.3 g, 4.65 mmol) in Et_2O/MeCN (13.5 mL/4.5 mL) at 0 °C was added imidazole (950 mg, 13.9 mmol), PPh_3 (1.83 g, 6.98 mmol) and I_2 (1.77 g, 6.98 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 1 h. After addition of pentane, the resulting mixture was filtered off on a sintered glass funnel filled with a pad of silica gel 1 cm thick, and washed with EtOAc. After evaporation of the filtrate under reduced pressure, the residue was purified by flash chromatography (Cyclohexane/Ethyl acetate, 70:30) to afford compound **29b** (1.05 g, 58%) as a colorless viscous oil. ^{1}H NMR ($CDCl_3$) δ: 7.84 (m, 2H, $2 \times$ H-phenyl), 7.71 (m, 2H, $2 \times$ H-phenyl), 3.90 (t, 2H, J = 5.7 Hz, CH_2), 3.75 (t, 2H, J = 5.7 Hz, CH_2), 3.59–3.69 (m, 6H, $3 \times CH_2$), 3.16 (td, 2H, J = 6.9 Hz, J = 1.0 Hz, CH_2) ppm.

*2.2.22. N^6,N^6-Bis(tert-butoxycarbonyl)adenine (**30**) [28]*

To a solution of adenine (1.35 g, 10 mmol) and DMAP (122 mg, 1 mmol) in THF (50 mL) under argon was added Boc_2O (9.38 g, 43 mmol). The reaction was stirred 5 h at room temperature. After removal of the solvent, EtOAc (400 mL) was added and the solution was washed with HCl 1 N (30 mL) and then with NaCl (3 × 100 mL). After drying over Na_2SO_4, filtration and removal of the solvents under reduced pressure, the residue was dissolved in methanol (100 mL) and $NaHCO_3$ sat. (45 mL). The reaction was then stirred for 1h30 at 50 °C and after removal of the solvent, water (100 mL) was added. The aqueous phase was then extracted with $CHCl_3$ (2 × 300 mL) and, after drying over Na_2SO_4, the solvent was removed under reduced pressure. The resulting crude product was purified by flash chromatography (cyclohexane/AcOEt, 10:90) to afford compound **30** (2.44 g, 73%) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 13.69 (s, 1H, NH), 8.80 (s, 1H, H-2), 8.63 (s, 1H, H-8), 1.36 (s, 18H, C(C**H**$_3$)$_3$) ppm.

*2.2.23. N-{2-[2-(N^6,N^6-Bis(tert-butoxycarbonyl)adenin-9-yl)ethoxy] ethyl}phtalimide (**31a**)*

To a solution of protected adenine **30** (295 mg, 0.88 mmol) in DMF (10 mL) was added NaH (60% in mineral oil, 42 mg, 1.05 mmol). The reaction mixture was stirred at 70 °C for one hour and then compound **29a** (364 mg, 1.05 mmol) was added and the reaction mixture was stirred for 4 h at 70 °C. After addition of water (20 mL) and EtOAc (30 mL) and decantation, the aqueous phase was extracted with EtOAc (2 × 30 mL) and the organic phase was dried over Na_2SO_4. After filtration and removal of the solvents under reduced pressure, the residue was purified by flash chromatography (cyclohexane/AcOEt, 30:70) to afford compound **31a** (298 mg, 61%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 8.83 (s, 1H, H-2), 8.38 (s, 1H, H-8), 7.86 (m, 2H, 2 × H-phenyl), 7.72 (m, 2H, 2 × H-phenyl), 4.46 (2H, t, J = 5.0 Hz, CH_2), 3.88 (2H, t, J = 5.4 Hz, CH_2), 3.82 (2H, t, J = 4.8 Hz, CH_2), 3.68 (2H, t, J = 5.3 Hz, CH_2), 1.46 (s, 18H, C(C**H**$_3$)$_3$) ppm; ^{13}C NMR ($CDCl_3$) δ: 168.32 (C), 153.29 (C), 151.92 (CH), 150.59 (C), 150.05 (C), 146.11 (CH), 134.20 (CH), 132.04 (C), 128.24 (C), 123.51 (CH), 83.86 (C), 68.50 (CH_2), 43.93 (CH_2), 37.25 (CH_2), 29.78 (CH_2), 27.90 (CH_3).

*2.2.24. 9-[2-(2-Aminoethoxy)ethyl]adenine (**32a**)*

To a solution of compound **31a** (298 mg, 0.54 mmol) in CH_2Cl_2 (5 mL) was added trifluoroacetic acid (1.5 mL, 20.2 mmol). The reaction mixture was then stirred at room temperature for 16 h. After removal of the solvent under reduce pressure, EtOAc was added and the precipitate was filtered to afford the fully Boc deprotected compound (197 mg, 78%, trifluoroacetate salt) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 8.66 (s, 2H, NH_2), 8.31 (s, 1H, H-2), 8.23 (s, 1H, H-8), 7.81 (m, 4H, H-phenyl), 4.32 (t, 2H, J = 4.8 Hz, CH_2), 3.78 (t, 2H, J = 5.1 Hz, CH_2), 3.68 (t, 2H, J = 4.8 Hz, CH_2), 3.62 (t, 2H, J = 5.1 Hz, CH_2) ppm.

To a solution of the fully Boc deprotected compound (197 mg, 0.42 mmol) in absolute ethanol (5 mL) was slowly added methylhydrazine (269 μL, 5.07 mmol). The reaction mixture was then stirred at 70 °C for 17 h. After removal of the solvents under reduced pressure, the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 80:20 with 2% of Et_3N) to afford compound **32a** (87 mg, 73% from **31a**) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 8.13 (s, 1H, s, H-2), 8.11 (s, 1H, H-8), 7.17 (broad s, 2H, NH_2), 4.30 (t, 2H, J = 5.3 Hz, CH_2), 3.75 (t, 2H, J = 5.3 Hz, CH_2), 3.38 (m, 4H, 2 × CH_2), 2.64 (broad s, 2H, NH_2) ppm.

*2.2.25. N-{2-[2-(2-(N^6,N^6-Bis(tert-butoxycarbonyl)adenin-9-yl) ethoxy)ethoxy]ethyl}phtalimide (**31b**)*

To a solution of protected adenine **30** (500 mg, 1.49 mmol) in DMF (8 mL) was added NaH (60% in mineral oil, 65 mg, 1.64 mmol). The reaction mixture was stirred one hour at 70 °C and then compound **29b** (580 mg, 1.49 mmol) was added. The resulting mixture was then stirred at 70 °C for 19 h. After addition of water (20 mL) and EtOAc (30 mL) and decantation, the aqueous phase was extracted with EtOAc (3 × 20 mL) and the organic phase was dried over Na_2SO_4. After filtration and removal of the solvents under reduced pressure, the residue was purified by flash chromatography (cyclohexane/AcOEt, 10:90) to afford compound **31b** (412 mg, 46%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 8.83 (s, 1H, s, H-2), 8.31 (s, 1H, H-8), 7.85 (m, 2H, 2 × H-phenyl), 7.71 (m, 2H, 2 × H-phenyl), 4.41 (2H, t, J = 5.1 Hz, CH_2), 3.90 (2H, t, J = 5.7 Hz, CH_2), 3.79 (2H, t, J = 4.8 Hz, CH_2), 3.71 (2H, t, J = 5.7 Hz, CH_2), 3.57 (m, 4H, 2 × CH_2), 1.45 (s, 18H, C(C**H**$_3$)$_3$) ppm.

*2.2.26. 9-{2-[2-(2-Aminoethoxy)ethoxy]ethyl}adenine (**32b**)*

Compound **32b** according to the same procedure as for compound **32a**, scale: **31b** (412 mg, 0.69 mmol), CH_2Cl_2 (7 mL), trifluoroacetic acid (1.92 mL, 25.8 mmol) reaction time 16 h. To a solution of the obtained crude fully Boc deprotected compound (458 mg) in absolute ethanol (9 mL) was slowly added methylhydrazine (572 μL, 10.77 mmol). The reaction mixture was then stirred at 70 °C for 24 h. After removal of the solvents under reduced pressure, the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 80:20 with 2% of Et_3N) to afford compound **32b** (178 mg, 97% from **31b**) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 1H, H-2), 8.09 (s, 1H, H-8), 7.81 (s, 2H, disappears after D_2O addition, NH_2), 4.29 (t, 2H, J = 5.3 Hz, CH_2), 3.78 (t, 2H, J = 5.4 Hz, CH_2), 3.45 (m, 4H, 2 × CH_2), 3.31 (t, 2H, J = 5.8 Hz, CH_2), 2.64 (t, 2H, J = 5.8 Hz, CH_2) ppm; ^{13}C NMR (DMSO-d_6) δ: 173.12 (C), 155.85 (C), 152.28 (CH), 149.44 (C), 141.13 (CH), 71.69 (CH_2), 69.41 (CH_2), 69.37 (CH_2), 68.19 (CH_2), 42.63 (CH_2), 41.03 (CH_2) ppm.

*2.2.27. Androst-4-en-3-one-17β-carboxylic acid (**33**) [29]*

A sodium hypobromite solution was prepared by slow addition of bromine (730 μL, 14.2 mmol) to a solution of sodium hydroxide (1.205 g, 30.1 mmol) in distilled water (5 mL) under argon atmosphere. This first mixture was stirred for 1 h to provide an orange solution. In a separate flask, a solution of sodium hydroxide (625 mg, 15.6 mmol) in distilled water (5 mL) was added to a solution of progesterone (1.00 g, 3.18 mmol) in *tert*-butanol (10 mL) at room temperature. This second mixture was stirred for 1 h at room temperature then cooled in an ice bath before being treated with the previously prepared sodium hypobromite solution. The resulting mixture was vigorously stirred at 0 °C for 3 h then the reaction was quenched with an aqueous solution of sodium sulfite (2.1 M, 1.5 mL). Stirring was continued overnight at room temperature. The mixture was concentrated *in vacuo* and extracted with toluene. The combined organic phases were acidified with concentrated HCl until pH 0. The resulting precipitate was filtered off and recristallised from methanol/water (4:1) to give compound **33** as a white solid (0.570 g, 57 % yield). Mp (dec.); ^{1}H NMR (DMSO-d_6) δ: 5.62 (s, 1H, H-4), 2.40–0.80 (m, 17H), 1.14 (s, 3H, CH_3), 0.67 (s, 3H, CH_3) ppm.

*2.2.28. N-succinimidyl ester of androst-4-en-3-one-17β-carboxylic acid (**34**) [30]*

To a solution of *N*-hydroxysuccinimide (110 mg, 0.96 mmol) and compound **33** (300 mg, 0.95 mmol) in anhydrous THF (6 mL) was added a solution of *N,N′*-dicyclohexylcarbodiimide (DCC, 195 mg, 0.95 mmol) in anhydrous THF (3 mL). The reaction mixture was stirred overnight at room temperature. The resulting mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 99:1) followed by recristallisation from methanol/water to give compound **34** as a white solid (216 mg, 55 % yield). Mp = 233–236 °C; ^{1}H NMR (DMSO-d_6) δ: 5.64 (s, 1H, H-4),

2.81 (m, 4H, 2 × CH_2-succinimide), 2.50–0.90 (m, 20H), 1.16 (s, 3H, CH_3), 0.78 (s, 3H, CH_3) ppm.

*2.2.29. 17β-{[2-(2-(6-Aminopurin-9-yl)ethoxy)ethyl] carbamoyl} androst-4-en-3-one (**1h**)*

To a solution of compound **34** (47 mg, 0.11 mmol) and compound **32a** (25 mg, 0.11 mmol) in dry DMF (2 mL) was added *N,N*-diisopropylethylamine (20 μL, 0.17 mmol). The resulting mixture was stirred at 35 °C during 20 h. Water (20 mL) and ethyl acetate (15 mL) were then added and, after extraction and decantation, the aqueous phase was extracted with ethyl acetate (2 × 15 mL). The organic phases were then dried over Na_2SO_4, filtred and the solvents were removed *in vacuo*. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 90:10) to give compound **1h** as a white solid (36 mg, 61% yield). Mp = 202–203 °C; ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 1H, H-2′), 8.08 (s, 1H, H-8′), 7.33 (t, 1H, *J* = 5.7 Hz, NHCO), 7.17 (s, 2H, NH_2), 5.62 (s, 1H, H-4), 4.28 (t, 2H, *J* = 5.3 Hz, CH_2), 3.76 (t, 2H, *J* = 5.3 Hz, CH_2), 3.40 (m, 2H, CH_2), 3.28 (m, 1H), 3.06 (m, 1H), 2.43–0.84 (m, 20H), 1.12 (s, 3H, CH_3-19), 0.54 (s, 3H, CH_3-18) ppm; ^{13}C NMR (DMSO-d_6) δ: 198.08 (C), 171.65 (C), 171.00 (C), 155.92 (C), 152.35 (CH), 149.52 (C), 141.11 (CH), 123.15 (CH), 118.57 (C), 69.04 (CH_2), 68.10 (CH_2), 55.42 (CH), 54.88 (CH), 53.24 (CH), 43.22 (C), 42.72 (CH_2), 38.33 (CH_2), 38.20 (C), 37.36 (CH_2), 35.12 (CH_2), 34.97 (CH), 33.62 (CH_2), 32.00 (CH_2), 31.69 (CH_2), 24.20 (CH_2), 23.12 (CH_2), 20.31 (CH_2), 16.87 (CH_3), 13.21 (CH_3) ppm; HRMS (ESI) *m/z*: calc for $C_{29}H_{40}N_6O_3$ + H: 521.3235, found: 521.3220.

*2.2.30. 17β-{[2-(2-[2-(6-Aminopurin-9-yl)ethoxy]ethoxy)ethyl] carbamoyl}androst-4-en-3-one (**1i**)*

To a solution of compound **34** (104 mg, 0.25 mmol) and compound **32b** (67 mg, 0.25 mmol) in dry DMF (2 mL) was added diisopropylethylamine (20 μL, 0.17 mmol). The resulting mixture was stirred at 35 °C during 20 h. Water (20 mL) and ethyl acetate (15 mL) were then added and, after extraction and decantation, the aqueous phase was extracted with ethyl acetate (2 × 15 mL). The organic phases were then dried over Na_2SO_4, filtred and the solvents were removed *in vacuo*. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 90:10) to give 157 mg of a yellow paste. This fraction was further purified (removal of DMF traces) using TLC plates, eluting one time with CH_2Cl_2/MeOH, 90:10 and two times with CH_2Cl_2/MeOH, 95:5. The silica was rinsed with AcOEt and then with anhydrous ethanol. Evaporation of the ethanolic fraction gave compound **1i** as a yellow paste (39 mg, 27% yield). ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 1H, H-2′), 8.10 (s, 1H, H-8′), 7.37 (broad t, 1H, *J* = 5.4 Hz, NHCO), 7.19 (s, 2H, NH_2), 5.62 (s, 1H, H-4), 4.29 (t, 2H, *J* = 5.4 Hz, CH_2), 3.78 (t, 2H, *J* = 5.3 Hz, CH_2), 3.60 (m, 1H), 3.50 (m, 4H, 2 × CH_2), 3.06 (m, 1H), 2.44–0.84 (m, 22H), 1.10 (s, 3H, CH_3-19), 0.58 (s, 3H, CH_3-18) ppm; ^{13}C NMR (DMSO-d_6) δ: 198.02 (C), 171.61 (C), 170.96 (C), 155.93 (C), 152.34 (CH), 149.49 (C), 141.12 (CH), 123.14 (CH), 118.56 (C), 69.43 (CH_2), 69.37 (CH_2), 69.25 (CH_2), 68.30 (CH_2), 55.36 (CH), 54.87 (CH), 53.23 (CH), 43.21 (C), 42.60 (CH_2), 38.36 (CH_2), 38.16 (C), 37.31 (CH_2), 35.09 (CH_2), 34.96 (CH), 33.61 (CH_2), 31.99 (CH_2), 31.67 (CH_2), 24.19 (CH_2), 23.09 (CH_2), 20.36 (CH_2), 16.84 (CH_3), 13.24 (CH_3) ppm; HRMS (ESI) *m/z*: calc for $C_{31}H_{44}N_6O_4$ + H: 565.3497, found: 565.3488.

2.2.31. Oxidation of progesterone by chloranil

To a solution of progesterone (2.44 g, 7.8 mmol) in *tert*-butanol (170 mL) was added chloranil (4.6 g, 18.7 mmol) at room temperature. The reaction mixture was heated to reflux for one hour then cooled down to room temperature and filtered. The precipitate was washed with chloroform. Combined filtrates were concentrated *in vacuo* and the residue was diluted in chloroform (200 mL). Organic phase was successively washed with water (3 × 20 mL), 1.2 M NaOH aqueous solution (4 × 20 mL) then with water (4 × 20 mL). The organic phase was dried over Na_2SO_4, filtered through Whatman #4 paper and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (ACOEt/cyclohexane, 1:2) to give compound **35a** as a white solid (1.23 g, 50% yield) and trace amounts of epimer **35b** (0.01 g, 4% yield).

*2.2.31.1. Δ^6-Progesterone (**35a**)[9,31].* Mp = 144–145 °C (Litt 144–145 °C) [9]; ^{1}H NMR ($CDCl_3$) δ: 6.10 (m, 2H, H-6 et H-7), 5.67 (s, 1H, H-4), 2.64–2.51 (m, 2H), 2.42 (m, 1H), 2.29–1.10 (m, 13H), 2.12 (s, 3H, CH_3-21), 1.10 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.15 (C-20), 199.58 (C-3), 163.53 (C-5), 140.64 (CH-7), 128.26 (CH-6), 123.92 (CH-4), 63.37 (CH-17), 53.79 (CH), 50.68 (CH), 44.84 I, 38.69 (CH_2), 37.72 (CH), 36.16 I, 34.03 (2 × CH_2), 31.64 (CH_3-21), 24.02(CH_2), 23.02 (CH_2), 20.76 (CH_2), 16.42 (CH_3-19), 13.39 (CH_3-18) ppm.

*2.2.31.2. 17α-Δ^6-Progesterone (**35b**).* Mp = 137–139 °C. ^{1}H NMR ($CDCl_3$) δ: 6.10 (dd, 1H, *J1* = 9.8 Hz, *J2* = 1.5 Hz, H-6), 6.05 (dd, 1H, *J1* = 9.8 Hz, *J2* = 2.5 Hz, H-7), 5.62 (s, 1H, H-4), 2.82 (dd, 1H, *J1* = 8.3 Hz, *J2* = 2.0 Hz, H-17), 2.60–2.42 (m, 1H), 2.60–2.42 (m, 1H), 2.39–2.29 (m, 1H), 2.15–1.10 (m, 13H), 2.10 (s, 3H, CH_3-21), 1.06 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 212.45 (C-20), 199.48 (C-3), 163.59 (C-5), 141.28 (CH-7), 127.87 (CH-6), 123.69 (CH-4), 60.77 (CH-17), 50.05 (CH), 47.48 (CH), 46.33 I, 37.79 (CH), 36.03 I, 34.92 (CH_2), 33.96 (CH_2), 33.92 (CH_2), 32.95 (CH_3-21), 25.29 (CH_2), 24.48 (CH_2), 20.62 (CH_3-18 + CH_2), 16.36 (CH_3-19) ppm; MS (ESI): *m/z* (%) 647 (100) $[2M+Na]^+$, 335 (18) $[M+Na]^+$, 313 (65) $[M+H]^+$; HRMS (ESI): *m/z*: calc for $C_{21}H_{28}O_2$ + H: 313.2162, found: 313.2154.

*2.2.32. Progesterone-7α-carbonitrile (**36**)*

A solution of Et_2AlCN (1 M in toluene, 16 mL, 16 mmol) was added to a solution of Δ6-progesterone (**35a**) (1.23 g, 3.9 mmol) in anhydrous THF (20 mL) at room temperature. After 2 h stirring, the mixture was cooled down in an ice bath and a 0.5 M NaOH aqueous solution (80 mL) was added. Aqueous phase was extracted by CH_2Cl_2 and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography on silica gel (cyclohexane/AcOEt, 1:1) to give compound **36** as a white solid (1.17 g, 88% yield). Mp = 164–165 °C. ^{1}H NMR ($CDCl_3$) δ: 5.87 (d, 1H, H-4), 3.01 (ddd, *J* = 5.0 Hz, *J* = 4.3 Hz, *J* = 2.2 Hz, 1H, H-7), 2.71–1.16 (m, 18H), 2.13 (s, 3H, CH_3-21), 1.19 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18) ppm; ^{13}C ($CDCl_3$) δ: 208.85 (C-20), 198.37 (C-3), 163.13 (C-5), 127.26 (CH-4), 118.96 (CN), 63.08 (CH-17), 52.82 (CH), 48.72 (CH), 43.97 (C), 38.34 (C), 37.88 (CH_2), 36.77 (CH), 35.37 (CH_2), 34.96 (CH_2), 33.90 (CH_2), 32.96 (CH), 31.63 (CH_3-21), 23.90 (CH_2), 22.94 (CH_2), 20.90 (CH_2), 17.49 (CH_3-19), 13.39 (CH_3-18) ppm; MS(ESI): *m/z* (%) 701 (42) $[2M+Na]^+$, 362 (100) $[M+Na]^+$, 340 (65) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{22}H_{29}NO_2$ + H: 340.2271; found: 340.2281.

*2.2.33. Progesterone-7α-carboxylic acid (**38**)*

A solution of DIBAL-H (1 M in toluene, 20.7 mL, 20.7 mmol) was added dropwise to a solution of **36** (1.17 g, 3.4 mmol) in toluene (80 mL) at −40 °C. After 2 h stirring at −40 °C, the reaction mixture was quenched with methanol (15 mL) and a 1N HCl aqueous solution (30 mL). Aqueous phase was extracted with AcOEt. Combined organic phases were washed with water, dried over Na_2SO_4, filtered through Whatman #4 paper and concentrated *in vacuo* to give compound **37** as a white solid (1.2 g) which was used in the next step without further purification.

Jones' reagent (8N, 4.32 mL, 34.6 mmol) was added dropwise to a solution of **37** (1.2 g, 3.5 mmol) in acetone (60 mL) at −15 °C. The reaction mixture was stirred for 30 min. then excess of Jones' reagent was hydrolyzed with MeOH (40 mL). After concentration *in*

vacuo, the residue was partitioned between AcOEt and water. Aqueous phase was extracted with AcOEt. The combined organic extracts were successively washed with brine and a saturated $NaHCO_3$ aqueous solution. The combined aqueous extracts were cooled down by an ice bath then they were acidified by a concentrated HCl solution to pH 3. After extraction with AcOEt, the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give compound **38** (830 mg, 67% overall yield from **36**) as a white solid. Mp = 224–226 °C; ^{1}H NMR (DMSO-d_6) δ: 12.00 (bs, 1H, COOH), 5.74 (s, 1H, H-4), 2.84 (bs, 1H), 2.74–2.31 (m, 5H), 2.28–1.13 (m, 13H), 2.12 (s, 3H, CH_3-21), 1.20 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18) ppm; ^{13}C NMR (DMSO-d_6) δ: 208.53 (C-20), 197.74 (C-3), 174.46 (COOH), 169.35 (C-5), 124.39 (CH-4), 62.40 (CH-17), 51.93 (CH), 45.71 (CH), 43.57 I, 42.96 (CH), 37.92 I, 37.64 (CH_2), 36.23 (CH), 35.17 (CH_2), 34.79 (CH_2), 33.67 (CH_2), 31.18 (CH_3-21), 23.65 (CH_2), 22.21 (CH_2), 20.74 (CH_2), 17.33 (CH_3-19), 12.76 (CH_3-18) ppm; MS (ESI): *m/z* (%) 381 (100) $[M+Na]^+$, 359 (67) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{22}H_{30}NO_4$ + H: 359.2222; found: 359.2217.

2.2.34. General procedure for coupling reactions between acid **38** and adenine derivatives **17, 23–26**

DCC (58 mg, 0.28 mmol) and *N*-hydroxysuccinimide (32 mg, 0.28 mmol) were successively added to a solution of acid **38** (100 mg, 0.28 mmol) in anhydrous DMF (4 mL). The mixture was stirred overnight at room temperature. Then, adenine derivative (1.2 eq.) and *N,N*-diisopropylethylamine (DIEA) (720 μL, 0.41 mmol) were added. Stirring was continued for 4 h. After concentration *in vacuo*, the residue was partitioned between CH_2Cl_2 and water. Aqueous phase was thoroughly extracted with CH_2Cl_2. The combined organic extracts were concentrated and the residue was purified by flash chromatography on silica gel.

*2.2.34.1. Coupling reaction with adenine derivative (**17**).* Flash chromatography on silica gel (CH_2Cl_2/MeOH, 92:8) gave amide **2a** (Rf 0.35, 58 mg, 37% yield) as a white solid and succinamide **39a** (Rf 0.3, 47 mg, 31% yield) as a white solid.

*2.2.34.1.1. 7α-[4-(6-Aminopurin-9-yl)hexylcarbamoyl]pregn-4-en-3,20-dione (**2a**).* Mp = 132–133 °C; ^{1}H NMR ($CDCl_3$) δ: 8.32 (s, 1H, H-2′), 7.81 (s, 1H, H-8′), 5.89 (s, 2H, NH_2), 5.72 (t, 1H, *J* = 6.0 Hz, NH), 5.68 (s, 1H, H-4), 4.17 (t, 2H, *J* = 7.0 Hz, CH_2-6″), 3.16 (q, 2H, *J* = 6.0 Hz, NHC**H**$_2$), 2.73–0.99 (m, 27H), 2.09 (s, 3H, CH_3-21), 1.18 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.81 (C-20), 199.42 (C-3), 172.97 (CONH), 170.02 (C-5), 155.54 (C-6′), 152.81 (CH-2′), 149.77 (C-4′), 140.48 (CH-8′), 125.15 (CH-4), 119.20 (C-5′), 63.18 (CH-17), 52.15 (CH), 46.02 (CH), 44.77 (CH), 44.42 (C), 43.88 (CH_2), 38.80 (CH_2), 38.23 (C), 38.14 (CH_2), 37.72 (CH), 36.52 (CH_2), 35.39 (CH_2), 33.91 (CH_2), 31.59 (CH_3-21), 30.05 (CH_2), 26.27 (CH_2), 26.16 (CH_2), 24.98 (CH_2), 24.61 (CH_2), 22.81 (CH_2), 21.35 (CH_2), 17.92 (CH_3-19), 13.17 (CH_3-18) ppm; MS (ESI) *m/z* (%): 575 (100) $[M+H]^+$; HRMS (ESI) *m/z*: calcd for $C_{33}H_{47}N_6O_3$ + H: 575.3704; found: 575.3710.

*2.2.34.1.2. N-(3,20-dioxopregn-4-en-7-carbonyloxy)-N′-[4-(6-aminopurin-9-yl)hexyl] succinamide (**39a**).* Mp = 148–149 °C; ^{1}H NMR ($CDCl_3$) δ: 8.32 (s, 1H, H-2′), 7.86 (s, 1H, H-8′), 6.13 (m, 3H, NH_2, NHCO), 5.70 (s, 1H, H-4), 4.19 (t, 2H, *J* = 6.9 Hz, CH_2N), 3.19 (q, 2H, *J* = 6.4 Hz NHC**H**$_2$), 2.95 (bs, 1H, H-7), 2.74–1.10 (m, 26H), 2.54 (m, 4H, $COCH_2CH_2CO$), 2.07 (s, 3H, CH_3-21), 1.19 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 210.09 (C-20), 199.82 (C-3), 172.36 (CO), 170.83 (C-5), 168.12 (CO), 155.46 (C-6′), 152.60 (CH-2′), 149.62 (C-4′), 140.58 (CH-8′), 125.72 (CH-4), 119.05 (C-5′), 63.15 (CH-17), 51.77 (CH), 46.25 (CH), 44.26 (C), 43.94 (CH_2), 41.50 (CH), 39.28 (CH_2), 38.28 (C), 37.99 (CH_2), 37.26 (CH), 35.35 (CH_2), 35.09 (CH_2), 33.84 (CH_2), 31.48 (CH_3-21), 31.10 (CH_2), 28.96 (CH_2), 28.52 (CH_2), 26.13 (CH_2), 26.11 (CH_2), 24.02 (CH_2), 22.84 (CH_2), 21.14 (CH_2), 17.64 (CH_3-19), 13.01 (CH_3-18) ppm; MS (ESI) *m/z* (%): 690 (100) $[M+H]^+$, 410 (36); HRMS (ESI) *m/z*: calcd for $C_{37}H_{52}N_7O_6$ + H: 690.3974; found: 690.3974.

*2.2.34.2. Coupling reaction with adenine derivative (**23**).* Flash chromatography on silica gel (CH_2Cl_2/MeOH, 93:7) gave amide **2b** (Rf 0.35, 13 mg, 9% yield) as a white solid and succinamide **39b** (Rf 0.3, 7 mg, 5% yield) as a white solid.

*2.2.34.2.1. 7α-[4-(6-Aminopurin-9-yl)but-2-ynylcarbamoyl]pregn-4-en-3,20-dione (**2b**).* Mp = 215–216 °C; ^{1}H NMR ($CDCl_3$) δ: 8.32 (s, 1H, H-2′), 8.00 (s, 1H, H-8′), 6.24 (t, 1H, *J* = 4.8 Hz, NH), 6.19 (s, 2H, NH_2), 5.68 (s, 1H, H-4), 4.95 (bs, 2H, CH_2-4′), 4.02 (bs, 2H, $NHCH_2$), 2.79–1.05 (m, 19H), 2.08 (s, 3H, CH_3-21), 1.17 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.38 (C-20), 199.01 (C-3), 172.74 (CONH), 168.86 (C-5), 155.56 (C-6′), 153.03 (CH-2′), 149.57 (C-4′), 140.05 (CH-8′), 125.55 (CH-4), 119.47 (C-5′), 82.59 (C≡), 75.65 (≡C), 63.16 (CH-17), 52.06 (CH), 46.16 (CH), 44.53 (CH), 44.40 (C), 38.28 (C), 38.15 (CH_2), 37.81 (CH), 36.22 (CH_2), 35.50 (CH_2), 34.03 (CH_2), 33.49 (CH_2), 31.65 (CH_3-21), 29.19 (CH_2), 24.65 (CH_2), 22.88 (CH_2), 21.39 (CH_2), 17.96 (CH_3-19), 13.19 (CH_3-18) ppm; MS (ESI) *m/z* (%): 543 (100%) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{31}H_{38}N_6O_3$ + H: 543.3078; found: 543.3086.

*2.2.34.2.2. N-(3,20-dioxopregn-4-en-7-carbonyloxy)-N′-[4-(6-aminopurin-9-yl)but-2-ynyl] succinamide (**39b**).* Mp = 110–111 °C; ^{1}H NMR ($CDCl_3$) δ: 8.29 (s, 1H, H-2′), 8.09 (s, 1H, H-8′), 6.93 (bs, 1H, CONH), 6.51 (s, 2H, NH_2), 5.70 (s, 1H, H-4), 4.93 (bs, 2H, CH_2N), 4.04 (bs, 2H, $NHCH_2$), 2.97 (bs, 1H, H-7), 2.86–1.10 (m, 18H), 2.58 (m, 4H, CO-CH_2CH_2-CO), 2.07 (s, 3H, CH_3-21), 1.18 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.51 (C-20), 199.38 (C-3), 172.20 (CO), 171.28 (C-5), 170.50 (CO), 155.55 (C-6′), 152.88 (CH-2′), 149.45 (C-4′), 140.44 (CH-8′), 126.00 (CH-4), 119.31 (C-5'), 83.02 (C≡), 75.46 (≡C), 63.16 (CH-17), 51.91 (CH), 46.39 (CH), 44.27 (C), 41.64 (CH), 38.34 (C), 38.06 (CH_2), 37.40 (CH), 35.54 (CH_2), 35.16 (CH_2), 34.07 (CH_2), 33.73 (CH_2), 31.61 (CH_3-21), 29.83 (CH_2), 29.64 (CH_2), 24.17 (CH_2), 22.94 (CH_2), 21.22 (CH_2), 17.77 (CH_3-19), 13.15 (CH_3-18) ppm; MS (ESI) *m/z* (%): 680 (7) $[M+Na]^+$, 658 (100) $[M+H]^+$; HRMS (ESI): *m/z*: calcd for $C_{35}H_{43}N_7O_6$ + H: 658.3348; found: 658.3348.

*2.2.34.3. Coupling reaction with adenine derivative (**24**).* Flash chromatography on silica gel (CH_2Cl_2/MeOH, 90:10) to give amide (**2c**) (Rf 0.35, 33 mg, 22% yield) as a white solid and succinamide (**39c**) (Rf 0.3, 31 mg, 21% yield) as a white solid.

*2.2.34.3.1. (E)-7α-[4-(6-Aminopurin-9-yl)but-2-enylcarbamoyl]pregn-4-en-3,20-dione (**2c**).* Mp = 130–131 °C; ^{1}H NMR ($CDCl_3$) δ: 8.32 (s, 1H, H-2′), 7.81 (s, 1H, H-8′), 5.84 (t, 1H, *J* = 5.6 Hz, NH), 6.01 (s, 2H, NH_2), 5.80 (dt, 1H, *J* = 15.3 Hz, *J* = 5.7 Hz, CH=), 5.62 (s, 1H, H-4), 5.58 (dt, 1H, *J* = 15.3 Hz, *J* = 5.5 Hz, HC=), 4.78 (d, 2H, *J* = 5.7 Hz, CH_2-4″), 3.92–3.75 (m, 2H, $NHCH_2$), 2.67 (m, 1H, H-7), 2.64–1.10 (m, 19H), 2.08 (s, 3H, CH_3-21), 1.17 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.30 (C-20), 198.90 (C-3), 172.79 (CONH), 168.91 (C-5), 155.55 (C-6′), 152.96 (CH-2′), 149.95 (C-4′), 140.50 (CH-8′), 130.86 (HC=), 125.93 (=CH), 125.51 (CH-4), 119.63 (C-5′), 63.14 (CH-17), 52.12 (CH), 46.05 (CH), 44.87 (CH), 44.83 (CH_2), 44.38 (C), 40.37 (CH_2), 38.26 (C), 38.15 (CH_2), 37.80 (CH), 36.47 (CH_2), 35.49 (CH_2), 34.03 (CH_2), 31.65 (CH_3-21), 24.74 (CH_2), 22.81 (CH_2), 21.38 (CH_2), 17.99 (CH_3-19), 13.22 (CH_3-18) ppm; MS (ESI) *m/z* (%): 567 (14) $[M+Na]^+$, 545 (100) $[M+H]^+$; HRMS (ESI) *m/z*: calcd for $C_{31}H_{40}N_6O_3$ + H: 545.3235; found: 545.3239.

*2.2.34.3.2. N-(3,20-dioxopregn-4-en-7-carbonyloxy)-N′-[(E)-4-(6-aminopurin-9-yl)but-2-enyl] succinamide (**39c**).* Mp = 125–126 °C; ^{1}H NMR ($CDCl_3$) δ: 11.19 (bs, 1H, NHOCO), 8.28 (s, 1H, H-2′), 7.87 (s, 1H, H-8′), 6.77 (bs, 1H, CONH), 6.47 (s, 2H, NH_2), 5.82 (dt, 1H, *J* = 15.3 Hz, *J* = 5.8 Hz, HC=), 5.68 (s, 1H, H-4), 5.62 (dt, 1H,

J = 15.3 Hz, J = 5.1 Hz, =CH), 4.75 (d, 2H, J = 5.1 Hz, CH_2-4″), 3.83 (bs, 2H, NHC**H**$_2$), 2.94 (bs, 1H, H-7), 2.78–1.00 (m, 18H), 2.55 (m, 4H, $COCH_2CH_2CO$), 2.06 (s, 3H, CH_3-21), 1.17 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.64 (C-20), 199.44 (C-3), 172.38 (C), 171.35 (C), 170.58 (C), 167.65 (C), 155.68 (C-6′), 152.89 (CH-2′), 149.86 (C-4′), 140.89 (CH-8′), 131.35 (CH=), 126.05 (CH=), 125.55 (CH-4), 119.45 (C-5′), 63.25 (CH-17), 51.97 (CH), 46.47 (CH), 45.06 (CH_2), 44.35 (C), 41.70 (CH), 40.92 (CH_2), 38.42 (C), 38.15 (CH_2), 37.47 (CH), 35.63 (CH_2), 35.24 (CH_2), 34.15 (CH_2), 31.70 (CH_3-21), 24.23 (CH_2), 23.01 (CH_2), 21.30 (CH_2), 17.84 (CH_3-19), 13.24 (CH_3-18) ppm; MS (ESI) m/z (%): 660 (100) $[M+H]^+$; HRMS (ESI) m/z: calcd for $C_{35}H_{45}N_7O_6$ + H: 660.3504; found: 660.3513.

*2.2.34.4. Coupling reaction with adenine derivative (**25**).* Flash chromatography on silica gel (CH_2Cl_2/MeOH, 90:10) gave amide (**2d**) (Rf 0.35, 41 mg, 28% yield) as a white solid and succinamide (**39d**) (Rf 0.3, 40 mg, 26% yield) as a white solid.

*2.2.34.4.1. (Z)-7α-[4-(6-Aminopurin-9-yl)but-2-enylcarbamoyl]pregn-4-en-3,20-dione (**2d**).* Mp = 103–104 °C; ^{1}H NMR ($CDCl_3$) δ: 8.29 (s, 1H, H-2′), 7.87 (s, 1H, H-8′), 7.41 (t, 1H, J = 5.2 Hz, NH), 5.94 (s, 2H, NH_2), 5.79 (dd, 1H, J = 10.4 Hz, J = 7.8 Hz, CH=), 5.66 (m, 1H, =H), 5.67 (s, 1H, H-4), 4.89 (d, 2H, J = 7.3 Hz, CH_2-4″), 4.15–3.95 (m, 2H, NHC**H**$_2$), 2.68 (m, 1H), 2.61–1.13 (m, 18H), 2.09 (s, 3H, CH_3-21), 1.19 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.33 (C-20), 198.95 (C-3), 172.92 (CONH), 169.09 (C-5), 155.65 (C-6′), 152.39 (CH-2′), 149.76 (C-4′), 140.86 (CH-8′), 130.31 (HC=), 126.63 (=H), 125.42 (CH-4), 120.11 (C-5′), 63.21 (CH-17), 52.20 (CH), 46.11 (CH), 44.90 (CH), 44.46 (C), 41.07 (CH_2), 38.23 (CH_2), 37.85 (CH), 36.48 (CH_2), 35.51 (CH_2), 35.03 (CH_2), 34.06 (CH_2), 31.67 (CH_3-21), 24.60 (CH_2), 22.89 (CH_2), 21.46 (CH_2), 18.02 (CH_3-19), 13.25 (CH_3-18) ppm; MS (ESI) m/z (%): 567 (12) $[M+Na]^+$, 545 (100) $[M+H]^+$; HRMS (ESI) m/z: calcd for $C_{31}H_{40}N_6O_3$ + H: 545.3235; found: 545.3225.

*2.2.34.4.2. N-(3,20-dioxopregn-4-en-7-carbonyloxy)-N′-[(Z)-4-(6-aminopurin-9-yl)but-2-enyl] succinamide (**39d**).* Mp = 92–93 °C; ^{1}H NMR ($CDCl_3$) δ: 11.00 (bs, 1H, NHOCO), 8.32 (s, 1H, H-2′), 7.96 (s, 1H, H-8′), 7.59 (bs, 1H, CONH), 6.34 (s, 2H, NH_2), 5.82–5.60 (m, 2H, HC = CH), 5.71 (s, 1H, H-4), 4.90 (d, 2H, J = 7.3 Hz, CH_2-4″), 4.05 (t, 2H, J = 6.3 Hz, NHC**H**$_2$), 2.98 (bs, 1H, H-7), 2.70–1.10 (m, 18H), 2.62 (m, 4H, $COCH_2CH_2CO$), 2.06 (s, 3H, CH_3-21), 1.18 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.62 (C-20), 199.35 (C-3), 172.36 (C), 171.33 (C), 170.77 (C), 167.58 (C), 155.69 (C-6), 152.59 (CH-2′), 149.80 (C-4′), 141.18 (CH-8′), 130.49 (HC=), 126.60 (=H), 126.10 (CH-4), 119.79 (C-5′), 63.26 (CH-17), 51.92 (CH), 48.58 (CH), 46.35 (CH), 44.36 (C), 41.72 (CH), 41.02 (CH_2), 38.43 (C), 38.12 (CH_2), 37.55 (CH), 36.22 (CH_2), 35.16 (CH_2), 35.24 (CH_2), 34.17 (CH_2), 31.71 (CH_3-21), 24.29 (CH_2), 23.07 (CH_2), 21.29 (CH_2), 17.85 (CH_3-19), 13.25 (CH_3-18) ppm; MS (ESI) m/z (%): 660 (100) $[M+H]^+$; HRMS (ESI) m/z: calcd for $C_{35}H_{45}N_7O_6$ + H: 660.3504; found: 660.3510.

*2.2.34.5. Coupling reaction with adenine derivative (**26**).* Flash chromatography on silica gel (CH_2Cl_2/MeOH, 93:7) gave amide (**2e**) (Rf 0.35, 48 mg, 32% yield) as a white solid and succinamide (**39e**) (Rf 0.3, 35 mg, 23% yield) as a white solid.

*2.2.34.5.1. 7α-[4-(6-Aminopurin-9-yl)butylcarbamoyl]pregn-4-en-3,20-dione (**2e**).* Mp = 123–124 °C; ^{1}H NMR ($CDCl_3$) δ: 8.32 (s, 1H, H-2′), 7.82 (s, 1H, H-8′), 6.03 (t, 1H, J = 5.8 Hz, NH), 5.95 (s, 2H, NH_2), 5.66 (d, 1H, J = 1.2 Hz, H-4), 4.25 (t, 2H, J = 7.2 Hz, CH_2N), 3.27 (m, 2H, NHC**H**$_2$), 2.66 (m, 1H), 2.54–1.14 (m, 22H), 2.09 (s, 3H, CH_3-21), 1.18 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.30 (C-20), 198.88 (C-3), 173.13 (CONH), 169.06 (C-5), 155.47 (C-6′), 152.61 (CH-2′), 150.13 (C-4′), 140.83 (CH-8′), 125.46 (CH-4), 119.79 (C-5′), 63.16 (CH-17), 52.20 (CH), 46.10 (CH), 44.95 (CH), 44.40 (C), 43.40 (CH_2), 38.32 (CH_2), 38.26 (C), 38.20 (CH_2), 37.80 (CH), 36.54 (CH_2), 35.53 (CH_2), 34.04 (CH_2), 31.67 (CH_3-21), 27.63 (CH_2), 26.60 (CH_2), 24.75 (CH_2), 22.85 (CH_2), 21.41 (CH_2), 17.99 (CH_3-19), 13.25 (CH_3-18) ppm; MS (ESI) m/z (%): 569 (5) $[M+Na]^+$, 547 (100) $[M+H]^+$; HRMS (ESI) m/z: calcd for $C_{31}H_{42}N_6O_3$ + H: 547.3391; found: 547.3392.

*2.2.34.5.2. N-(3,20-dioxopregn-4-en-7-carbonyloxy)-N′-[4-(6-aminopurin-9-yl)butyl] succinamide (**39e**).* Mp = 144–145 °C; ^{1}H NMR ($CDCl_3$) δ: 8.31 (s, 1H, H-2′), 7.92 (s, 1H, H-8′), 6.60 (t, 1H, J = 5.5 Hz, CONH), 6.40 (s, 2H, NH_2), 5.69 (s, 1H, H-4), 4.21 (t, 2H, J = 7.0 Hz, CH_2N), 3.28 (m, 2H, NHC**H**$_2$), 2.97 (bs, 1H, H-7), 2.73–1.10 (m, 22H), 2.56 (m, 4H, $COCH_2CH_2CO$), 2.07 (s, 3H, CH_3-21), 1.18 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18) ppm; ^{13}C NMR ($CDCl_3$) δ: 209.44 (C-20), 199.20 (C-3), 172.46 (CO), 171.25 (C-5), 167.46 (CO), 155.52 (C-6′), 152.45 (CH-2′), 149.94 (C-4′), 141.17 (CH-8′), 125.99 (CH-4), 119.56 (C-5′), 63.16 (CH-17), 51.87 (CH), 46.32 (CH), 44.26 (C), 43.48 (CH_2), 41.60 (CH), 38.82 (CH_2), 38.33 (C), 38.05 (CH_2), 37.42 (CH), 35.56 (CH_2), 35.14 (CH_2), 34.08 (CH_2), 31.61 (CH_3-21), 27.42 (CH_2), 26.22 (CH_2), 24.16 (CH_2), 22.95 (CH_2), 21.20 (CH_2), 17.76 (CH_3-19), 13.15 (CH_3-18) ppm; MS (ESI) m/z (%): 662 (100) $[M+H]^+$, 382 (15); HRMS (ESI) m/z: calcd for $C_{35}H_{47}N_7O_6$ + H: 662.3661; found: 662.3675.

*2.2.35. Progesterone-7α-carboxylic acid methyl ester (**40**)*

To a stirred solution of acid **38** (54 mg, 150 μmol) in $CHCl_3$ (2 mL) at 0 °C, MeOH (20 μL, 490 μmol), DMAP (2 mg, 16 μmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI, 59 mg, 310 μmol) were added. After stirring overnight at room temperature, water was added and the mixture was extracted by CH_2Cl_2. The combined organic extracts were washed with water and brine, dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by preparative TLC (cyclohexane/AcOEt, 60:40) to give the ester **40** (Rf 0.3, 15 mg, 27% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 5.64 (s, 1H, H-4), 3.57 (s, 3H, $COOCH_3$), 2.77 (bs, 1H), 2.61–2.32 (m, 5H), 2.21–1.78 (m, 13H), 2.13 (s, 3H, CH_3-21), 1.14 (s, 3H, CH_3-19), 0.59 (s, 3H, CH_3-18) ppm; MS (ESI): m/z (%) 767 (32) $[2M+Na]^+$, 411 (68) $[M+K]^+$, 395 (100) $[M+Na]^+$, 373 (21) $[M+H]^+$.

2.3. Biological evaluation

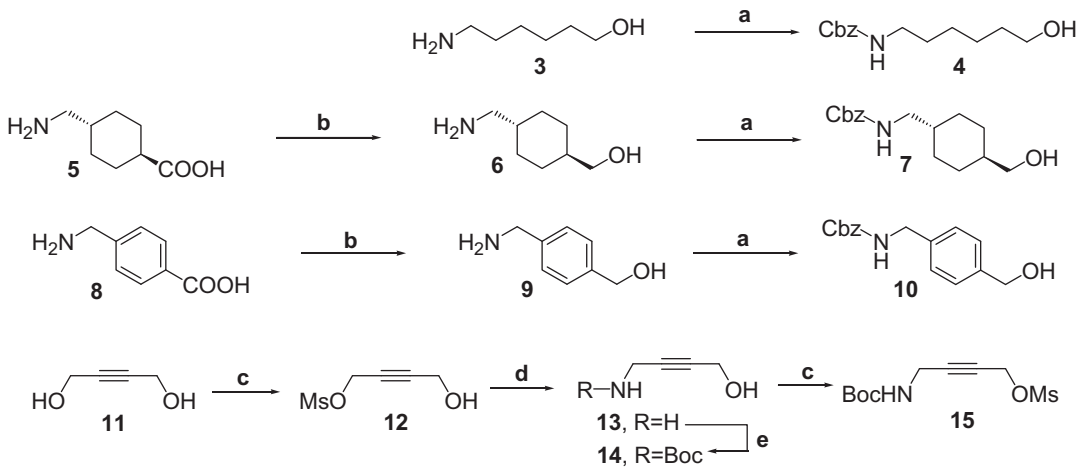
The inhibition of Pgp activity by the studied compounds was evaluated by their ability to prevent the efflux of daunorubicin in the K562/R7 drug-selected human cell line [30]. One million K562/R7 human leukemic cells expressing high levels of P-glycoprotein were incubated for 1 h at 37 °C in 1 mL of RPMI 1640 medium containing a final concentration of 10 μM daunorubicin, in the presence or absence of inhibitor (at 10 or 50 μM). After incubation, the cells were then washed twice with ice-cold phosphate buffered saline (PBS) and kept on ice until analysis by flow cytometry on a FACS-Calibur (Becton–Dickinson Corp., Mountain View, CA). Progesterone was used as a positive control (mean inhibitory activity of 100%). Assays were performed in duplicate, with at least two separate experiments.

3. Results and discussion

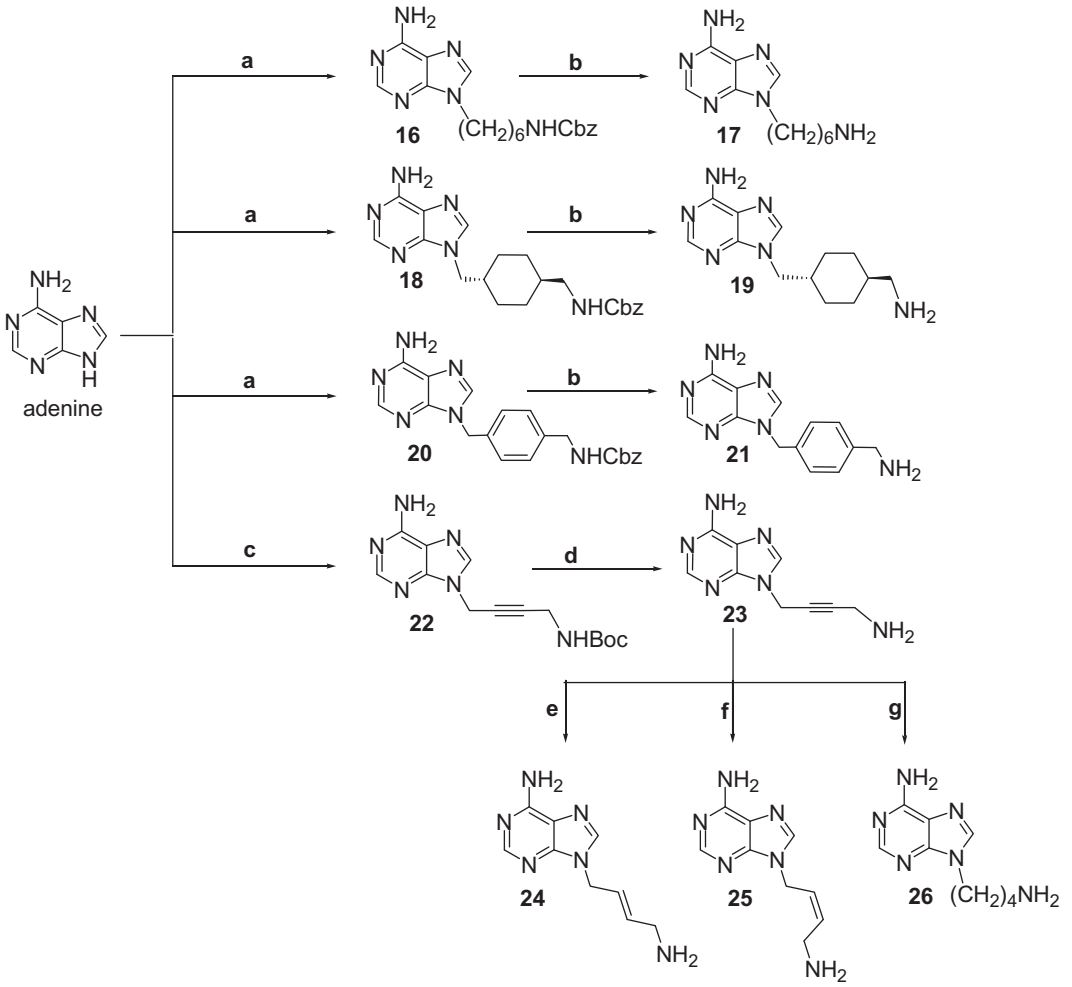
3.1. Chemistry

As their syntheses have not been previously described in detail [19], we report here the preparation of adenine-spacer-NH_2 derivatives **17**, **19**, **21** and **23**–**26**, obtained from the building blocks **4**, **7**, **10** and **15**, following the reaction pathways described in Schemes 1 and 2.

Of note, whatever the *N*-alkylating conditions of adenine we used, this reaction produced the *N*-9 alkylated isomer. As shown



Scheme 1. Reagents and conditions: (a) Na_2CO_3, CbzCl, H_2O, 0 °C to RT, overnight (79% for **4**; 70% for **7**; 90% for **10**); (b) $LiAlH_4$, THF, 0 °C to reflux, 3 h (70% for **6**; 28% for **9**); c) MsCl, Et_3N, THF, 0 °C to RT, overnight (45% for **12**; 76% for **15**); (d) NH_4OH, RT, 1 h then Dowex (76%); (e) Boc_2O, Et_3N, THF, RT, overnight (73%).



Scheme 2. Reagents and conditions: (a) PPh_3, DIAD, THF, **4** or **7** or **10** (41% for **16**, 49% for **18**, 18% for **20**); (b) H_2, Pd/C, MeOH or MeOH/AcOEt (100% for **17**, 71% for **19**, 77% for **21**); (c) K_2CO_3, DMF, **15** (75%); (d) (i) aq. HCl, (ii) Dowex 550A (hydroxide form) (80%); (e) $LiAlH_4$, THF (29%); (f) H_2, Lindlar catalyst, quinoline, MeOH (67%); (g) H_2, Pd/C, MeOH (90%).

in Fig. 4, regioselectivity was confirmed by NMR analysis including {^{1}H; ^{13}C} HMBC correlations recorded for compounds **16** and **24**, respectively obtained by Mistsunobu coupling reaction or nucleophilic substitution. In each case, long-distance coupling constants ^3J were measured on one hand, between the adenine amino proton (7.17 or 7.21 ppm) and the quaternary carbon C-5 (118.73 or 118.65 ppm) and on the other hand, between *N*-methylene proton H-1′ (4.11 or 4.73 ppm) and the other quaternary carbon C-4

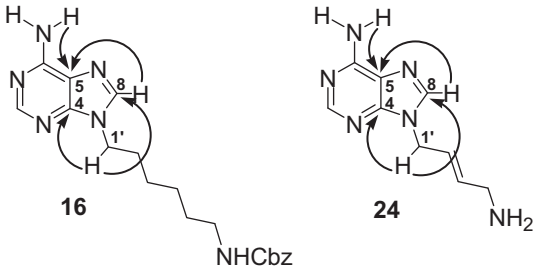


Fig. 4. Establishment of the regioselectivity of the *N*-alkylation of adenine by HMBC NMR sequence. ^{1}H NMR spectra were recorded in DMSO-d_6.

(149.54 or 149.32 ppm). Furthermore, the structure of alkylated isomers of adenine was also assigned by comparison with UV literature data [32]. The UV spectrum of *N*-9 substituted isomers in $CHCl_3$ showed one absorption maximum at 260 nm while a maximum at 270 nm is characteristic of *N*-7 substituted isomers.

Secondly, poly(ethyleneglycol)-modified adenine derivatives **32a,b** were prepared as described in Scheme 3. As preliminary studies have shown that the formation of the terminal amino group after various reduction conditions of the corresponding adenine-PEG azides (H_2/Pd-C, PPh_3, NaI/$CeCl_3$) [33] have failed to produce pure adenine–PEG–NH_2 derivative **32a**, we turned our attention to another method. This strategy involved an alkylation step of potassium phtalimide with commercially available chloroalcohols **27a,b**, followed by conversion of the alcohols **28a,b** into alkyl iodides **29a,b** using Corey's method (combination of PPh_3 and iodine, in the presence of imidazole). (See Scheme 4).

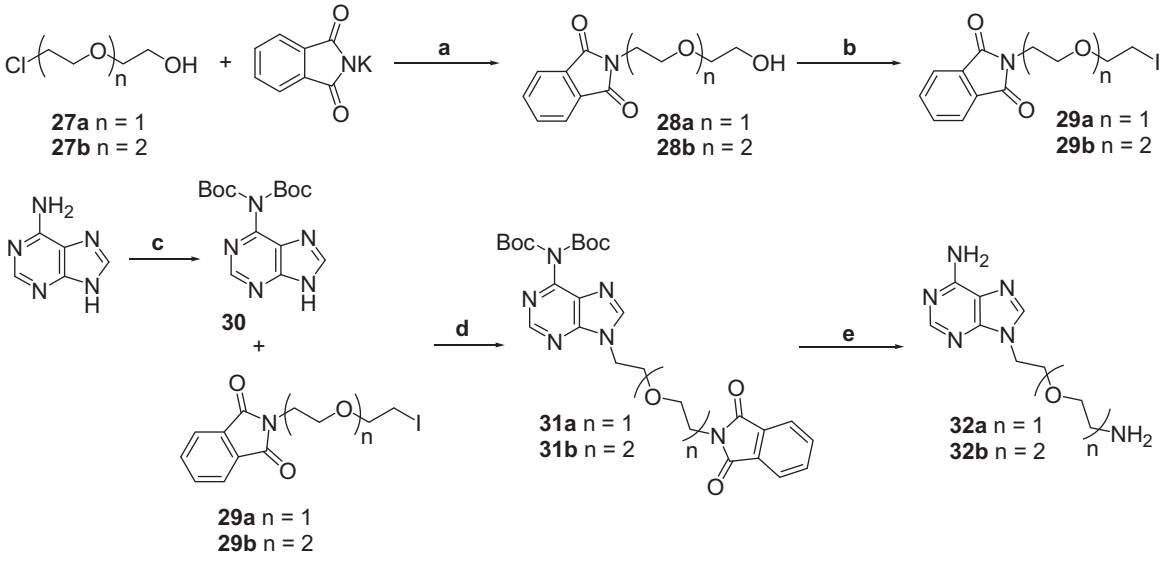
In a first attempt, the building blocks **29a,b** were coupled with adenine under nucleophilic substitution conditions affording the corresponding *N*-9-alkylated purine in low yields. This reaction was then applied to *N*-6 diBoc adenine **30** to get diprotected compounds **31a,b** in more satisfactory yields. Subsequent *N*-Boc deprotections were carried out in acidic conditions and the primary amino group was then recovered after hydrazinolysis of these phtalimide functionalized PEGs to get the expected products **32a,b** in good overall yields.

The methodology previously described to synthesize compounds **1a–g** [19] was applied to prepare PEG hybrids **1h,i**. The activated *N*-hydroxysuccinimidyl ester **34** [30] was allowed to react with the adenine derivatives **32a,b** in the presence of DIEA in DMF to afford the corresponding C20-progesterone-PEG-adenine hybrids **1h–i** in low to moderate yields.

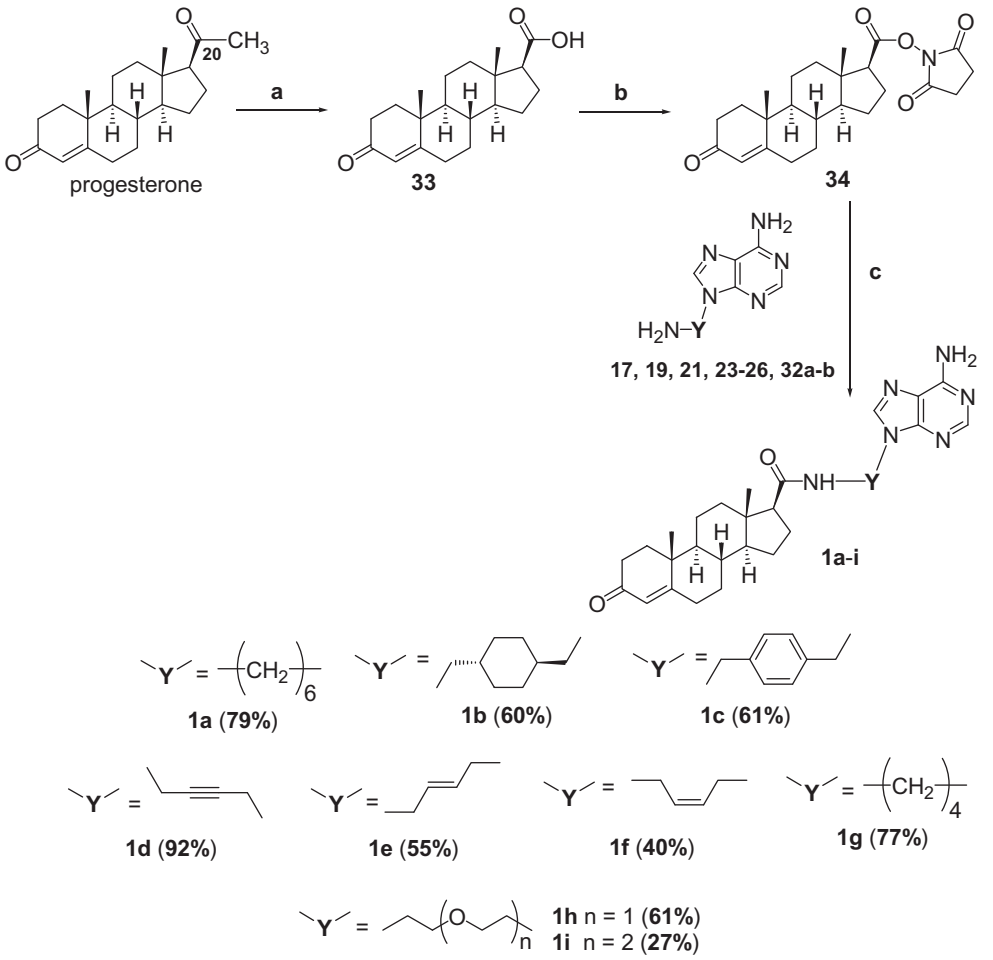
At the same time, we have synthesized a new series of hybrids whose linker arms were attached at the C7-position of the steroid nucleus. 7-Substituted progesterone derivatives are generally prepared by 1,6-addition of a nucleophile to Δ^6-progesterone **35a** [34]. The latter was obtained by oxidation of progesterone by tetrachloro-1,4-benzoquinone (chloranil) in refluxing *tert*-butanol according to a literature procedure [35] (Scheme 5). This reaction was monitored by UV spectroscopy after dilution of a reaction aliquot in absolute EtOH and carried out until the complete shift of the absorption maximum from 240 nm (characteristic of progesterone) to 280 nm (characteristic of 3-oxo-4,6-diene steroids). In our case, it is noteworthy that this oxidation step also led to traces of the 17α-epimer **35b** which made the reaction mixture more difficult to purify. The structure of **35b** was unambiguously established by comparison of its proton NMR spectrum in $CDCl_3$ to that of **35a**. Indeed, CH_3-18 and H17 proton signals of 17α-isomer (δ[CH_3-18] = 0.96 ppm, δ[H17] = 2.82 ppm) were significantly deshielded compared with the corresponding protons in 17β-isomer (δ[CH_3-18] = 0.70 ppm, δ[H17] = 2.55 ppm) [31].

With the aim of using an amide link as a connection function between the steroid moiety and the spacer, a carboxylic acid function had to be introduced in position 7 of progesterone. As described in Scheme 6, the first step was the stereoselective introduction of a cyano group at the 7α-position of the steroid framework in a high 88% yield, by 1,6-nucleophilic addition to the dienone **35a** in presence of an excess of diethyl aluminum cyanide [36]. Under these very mild conditions, no epimerization was detected. Stereochemistry was confirmed by proton NMR: the width of the H7-proton NMR peak (δ = 3.01 ppm) at half height was 8.30 Hz, which matched with an equatorial proton. Furthermore, it was shown in literature that conformationally rigid substrates favored axial attack of the cyanide anion [37].

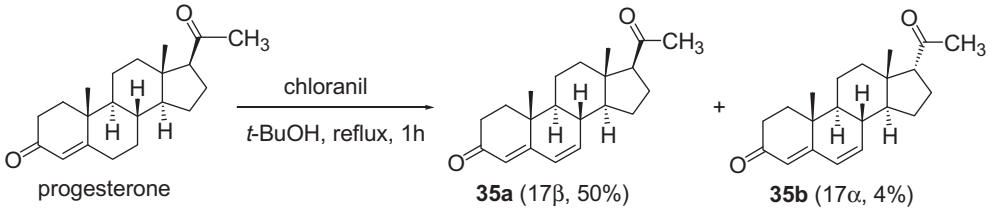
Treatment of nitrile **36** with diisobutyl aluminum hydride in anhydrous toluene at −40 °C induced the simultaneous reductions



Scheme 3. Reagents and conditions: (a) DMF, 130 °C, 2 h (85% for **28a**; 97% for **28b**); (b) PPh_3, I_2, imidazole, Et_2O, CH_3CN, 0 °C for 2 h then RT for 1 h (72% for **29a**; 58% for **29b**); (c) (i) Boc_2O, DMAP, THF, RT, 5 h (ii) $NaHCO_3$, MeOH, 50 °C, 1h30 (73% over the two steps) (d) NaH 60%, DMF, 70 °C, **29a** (5 h) or **29b** (20 h) (61% for **31a**; 46% for **31b**); (e) (i) TFA, CH_2Cl_2, RT, 16 h (ii) $MeNHNH_2$, abs. EtOH, 70 °C, (17 h, 73% for **32a**; 24 h, 97% for **32b**).



Scheme 4. Reagents and conditions: (a) (i) NaOH, Br_2, *t*-BuOH, 0 °C, 3 h (ii) Na_2SO_3, RT, overnight (57%); (b) *N*,*N*′-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (HOSu), THF, RT, overnight (55%) and (c) DIEA, DMF, overnight, RT (**1a**–**b**, **1d**–**g**) or 35 °C (**1c**, **1h**,**i**).



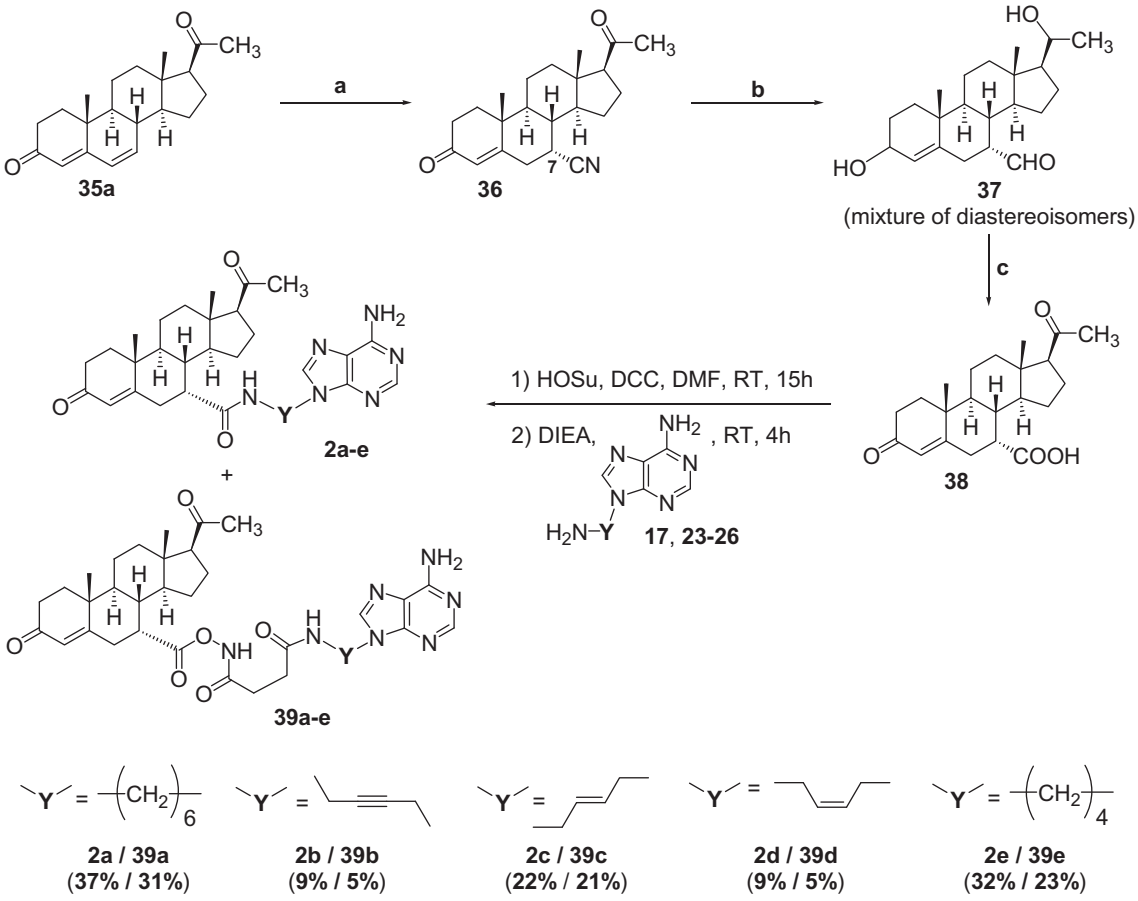
Scheme 5. Synthesis of Δ^6-progesterone.

of cyano group and both C3- and C20-ketones to afford the dihydroxy aldehyde **37** as a mixture of diastereoisomers. This mixture was easily oxidized without further purification, by an excess of Jones reagent (CrO_3, H_2SO_4) at low temperature to obtain carboxylic acid function at the position 7 and regenerate C3- and C20-ketones. Compound **38** was isolated as the sole isomer in good overall yield (29% from progesterone).

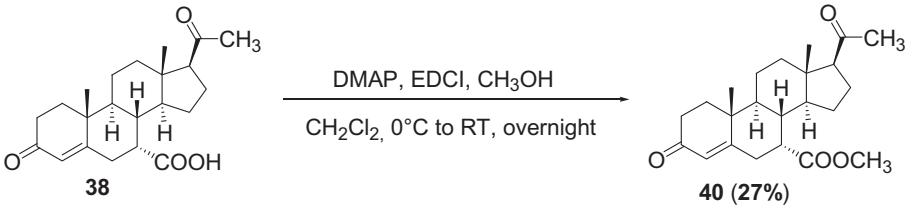
Finally, coupling reactions in C7 position were carried out in a slightly different manner as previously described for C20 hybrids. Because of the degradation of the activated *N*-hydroxysuccinimidyl ester during its purification, coupling reactions with five adenine-spacer-NH_2 derivatives (**17** and **23–26**) were performed in a one-pot sequential DCC/HOSu activation-amidation process. Somewhat surprisingly, the reaction of acid **38** with each of the amines led not only to amides **2a–e** but also to succinamides **39a–e** in a ratio close to 1:1. The formation of compounds **39a–e** might arise from nucleophilic attack of the amine at one of the carbonyl groups of the succinimide intermediate, thus leading to its ring-opening [38]. This side reaction might be favored by a sterically more hindered *N*-hydroxysuccinimidyl ester at C7 position of progesterone than at C20 position. Amides **2a–e** and succinamides **39a–e** were obtained in low to moderate total yields.

The esterification of acid **38** with methanol was also carried out (Scheme 7) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) and the water soluble coupling reagent, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI).

This simpler 7α-carboxymethyl derivative **40** was used as a secondary control: its inhibitory activity was compared to those obtained for C7-hybrids **2** and **39**.



Scheme 6. Reagents and conditions: (a) Et_2AlCN (1 M in toluene), THF-toluene, RT, 2 h (88%); (b) DIBAL-H (1 M in toluene), toluene, −40 °C, 2 h. (c) CrO_3, H_2SO_4, H_2O, acetone, −15 °C, 30 min (67% overall yield for 2 steps).



Scheme 7. Esterification of carboxylic acid **38**.

3.2. Biological evaluation

The efficiency of our hybrids progesterone-spacer-adenine **1**, **2** and **39** to inhibit Pgp-mediated cytotoxic drug efflux was evaluated by measuring the intracellular accumulation of daunorubicin by flow cytometry in K562/R7 human leukemic cells overexpressing Pgp (Table 1). Progesterone was used as positive control.

All new synthesized compounds (**1h,i, 2a–e**, **39a–e** and **40**) and the hybrid **1a** with a hexamethylene linker chain, which has previously shown the best inhibitory potency [19], were evaluated at 10 μM and at 50 μM as well, the latter concentration for which progesterone was known to reverse significantly the drug resistance in Pgp-overexpressing cells [17].

At a concentration of 10 μM, C20-PEG-hybrids (**1h,i**, entries 2 and 3), C7-hybrids (**2a–e**, **39a–e**, entries 4–8 and 9–13) and ester **40**, used as a secondary control (entry 14), showed an inhibitory activity similar to that of progesterone. At a concentration of 50 μM, the result for ester **40** confirmed that the substitution at the position 7α of the progesterone did not prevent the binding of the steroid nucleus to P-glycoprotein, as already described in literature [9,20]. On the other hand, at 50 μM, all the bivalent compounds are less efficient than progesterone, except hybrid **1a** (entry 1) which significantly enhanced the daunorubicin accumulation by 30 % after one-hour incubation at 37 °C.

As expected, both attachment point and nature of the linker play a fairly crucial role in the ability of these bivalent compounds to inhibit Pgp transport activity. Indeed, the connection of the hexamethylene linker at the position 7α of the progesterone was not favorable (entry 4). This could be explained by an incorrect orientation of the steroid in its binding pocket. Moreover, tether flexibility induced by the use of a PEG linker (**1i**) also led to less potent Pgp modulators compared to more rigid hexamethylene one (entries 1 and 3).

Table 1
Capacity of the synthesized derivatives to improve daunorubicin accumulation in K562/R7 human leukemic resistant cells [39].

Entry	Compound	Daunorubicin accumulation (% progesterone) [a,c]	Daunorubicin accumulation (% progesterone) [b,d]
1	**1a**	110.6 (±13.4)	133.2 (±18.1)
2	**1h**	94.0 (±6.9)	62.0 (±8.2)
3	**1i**	90.3 (±1.7)	65.9 (±9.4)
4	**2a**	102.1 (±5.7)	52.7 (±5.0)
5	**2b**	101.1 (±6.3)	54.4 (±5.4)
6	**2c**	90.1 (±3.6)	52.2 (±3.3)
7	**2d**	98.2 (±2.7)	51.8 (±1.2)
8	**2e**	96.0 (±5.6)	50.6 (±5.5)
9	**39a**	98.2 (±3.7)	54.8 (±5.3)
10	**39b**	97.6 (±7.8)	55.7 (±7.4)
11	**39c**	98.8 (±4.2)	55.5 (±5.9)
12	**39d**	95.4 (±6.4)	51.6 (±6.8)
13	**39e**	91.2 (±4.8)	54.1 (±7.1)
14	**40**	101.4 (±6.3)	81.9 (±10.3)

[a] Compounds were tested at a 10 μM concentration.
[b] Compounds were tested at a 50 μM concentration.
[c] Accumulation of daunorubicin (10 μM) in the presence of progesterone (10 μM) was considered as 100%. Daunorubicin accumulation in the presence of the tested compounds (10 μM) was expressed as percent of the accumulation in the presence of progesterone. Standard deviation is given in brackets.
[d] Accumulation of daunorubicin (10 μM) in the presence of progesterone (50 μM) was considered as 100%. Daunorubicin accumulation in the presence of the tested compounds (50 μM) was expressed as percent of the accumulation in the presence of progesterone. Standard deviation is given in brackets.

4. Conclusion

We have described herein the synthesis and the biological evaluation of 13 new progesterone–adenine hybrids. An efficient four-step procedure was developed from progesterone to prepare C7α derivatives in a stereocontrolled manner.

The efficiency of progesterone–adenine hybrids might greatly depend on two main factors: (i) the nature of the linker and (ii) its attachment point on the steroid skeleton. Further studies are necessary to determine if the use of longer poly-methylene or -ethyleneglycol chains as spacers connected at C20 or C7 position of progesterone improve the inhibitory efficiency of these bivalent compounds. Some analogs like flavonoid derivatives could mimic the adenine moiety of ATP and bind to Pgp with high affinity at NBD site. The use of such compounds instead of adenine should therefore also be considered [40].

Acknowledgements

ZM thanks the Ministère de l'Enseignement Supérieur et de la Recherche for a PhD fellowship.

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